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16. ABSTRACT

This report represents the results of a comprehensive series of toxicological studies designed to evaluate the health effects of the application of recycled water for drinking purposes. Water was prepared in a highly advanced domestic sewerage pilot plant. Some 400,000 liters of the finished water were concentrated down to a volume of 200 liters with a total organic carbon content of 700 mg/l. This concentrate was incorporated into a gel-type diet which was fed to mice. A total of 900 animals was included in the experimental program, which extended to 150 days. The mice were tested for growth, food intake, mutagenicity, mortality, blood physiology and biochemistry, and liver and nervous system functions. Ten tissues were screened for pathological effects. Only marginal changes were demonstrated in these areas.

In a second series of experiments, rodent and human cells were tested in vitro for general toxicity, mutagenicity, and carcinogenicity. Results for all three effects in the tissue cultures were positive. These effects were significantly increased by the presence of a liver activation system.

These results show that exposure for a limited time (20% of a lifespan) to the concentrated, recycled water (about 100-1000 times present human exposure) does not lead to physiological changes in mice. On the other hand, the positive results from the mutagenicity and carcinogenicity studies in tissue culture indicate a need for more studies in this area.

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EPA-600/1-78-068
December 1978

EVALUATION OF TOXIC EFFECTS OF ORGANIC CONTAMINANTS IN RECYCLED WATER

by

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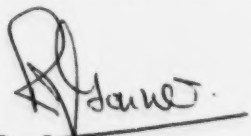
FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay among its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents found in the environment. In addition to valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

This report provides an assessment and discussion of the toxic effects of water recycled for drinking purposes. With a better understanding of the health effects, methods can be developed to produce recycled water suitable for human consumption.



R. J. Garner
Director
Health Effects Research Laboratory

ABSTRACT

This report represents the results of a comprehensive series of toxicological studies designed to evaluate the health effects of the application of recycled water for drinking purposes. Water was prepared in a highly advanced domestic sewerage pilot plant. Some 400,000 liters of the finished water were concentrated down to a volume of 200 liters with a total organic carbon content of 700 mg/liter. This concentrate was incorporated into a gel-type diet which was fed to mice. A total of 900 animals was included in the experimental program, which extended to 150 days. The mice were tested for growth, food intake, mutagenicity, mortality, blood physiology and biochemistry, and liver and nervous system functions. Ten tissues were screened for pathological effects. Only marginal changes were demonstrated in these tests.

In a second series of experiments, rodent and human cells were tested *in vitro* for general toxicity, mutagenicity, and carcinogenicity. Results for all three effects in the tissue cultures were positive. These effects were significantly increased by the presence of a liver activation system.

These results show that exposure for a limited time (20 percent of a lifespan) to the concentrated, recycled water (about 100-1000 times present human exposure) does not lead to physiological changes in mice. On the other hand, the positive results from the mutagenicity and carcinogenicity studies in tissue culture indicate a need for more studies in this area.

This report was submitted in fulfillment of Contract No. 68-03-2464 by Gulf South Research Institute under the sponsorship of the U.S. Environmental Protection Agency. This report covers a period from September 30, 1976 to May 31, 1978, and work was completed as of May 31, 1978.

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SECTION 1

INTRODUCTION

Water used to be one of our most plentiful natural resources. In recent years, however, we have discovered that water is no longer an abundant commodity in many places in this country and other countries in the world. Reasons for the reduced availability of water include population growth, increases in average water consumption for domestic and industrial purposes, and pollution. Although there are annual changes in water reservoirs, long-term trends show that shortage of water will become a problem in many places that are not now affected.

One approach to remedy this situation is conservation. Natural or unintentional reuse by humans has been known for many years. But the presence of hundreds of compounds in wastewater, among them known toxicants and carcinogens, necessitates a more cautious approach to wide-scale intentional reuse of treated wastewater. Treated wastewater can be used for a variety of purposes, including agriculture, industry, recreation and drinking purposes. Treated wastewater may be accessed for drinking water in several ways, such as recharge of ground water or direct supply with or without other sources of water. None of these approaches has been dealt with in a practical way. Obviously, drinking water should receive special toxicological consideration since it is a general daily commodity used throughout the lifetime and by everyone in the population.

In spite of extensive work in this area and the interest of many national and international bodies, relatively little effort has been devoted to the question of the health effects of renovated drinking water (1). Most of the work has been done on the technological aspects of the problem, some on the chemistry and biology, and almost none in the toxicological area. Although opinions of urgency and need for priority of this subject have been expressed in conferences worldwide (2,3), international collaboration is still occasional and unplanned. Studies that have been performed can be considered preliminary (4,5) and have been subject to inadequate water sample preparation, limitations on the extent of the toxicological studies, or both.

When the U.S. Environmental Protection Agency first launched efforts in this area, the necessity of finding a method to concentrate water was recognized. Many concentration systems are inappropriate because of selective removal or destruction of original compounds or addition of contaminants. The concentration method must also be adaptable to large scale operations. Reverse osmosis has been considered as the best choice in these respects (6).

When dealing with toxicological aspects of mixtures, two approaches can be taken (7). The first approach involves chemical analysis of the mixtures followed by individual toxicological assessment of each suspected chemical. This methodology has been used in air pollution and food contamination studies. In the second approach, the mixture is considered as one test compound. This approach was selected for this project for several reasons. (1) Hundreds of chemicals had already been identified in water. (2) Long-term toxic effects of most of these chemicals are unknown. (3) The unlimited possibilities of interactions of these compounds in the body, both synergistically and antagonistically, prevent the extrapolation of data from individual tests to the toxicological assessment of the mixture (water).

The following factors are involved in the assessment of the toxicological aspects of renovated water: (1) the presence of hundreds of compounds, some of them known toxicants, (2) lack of toxicological data on most of these compounds or at the low levels found in water (parts-per-billion range), (3) the possibility of synergistic and antagonistic interactions, and (4) general intake by the whole population from the early days of life (and even before birth) until old age.

These considerations led us to set up an extensive project. Long-term exposed mice were subjected to a battery of tests. The possible effects on the fetuses and the newborns were also examined. Because of special problems involved at present with mutagenic and carcinogenic assays in the whole animals, these tests were done mainly in tissue cultures.

SECTION 2

CONCLUSIONS

1. Some 400,000 liters of finished water from an advanced wastewater treatment plant was concentrated down to 200 liters with a final total organic carbon (TOC) content of 700 mg/liter. The recovery of the organic fraction was estimated to be only about 20 percent. A substantial fraction of the inorganic ions were eliminated or exchanged with other ions so the solution could be balanced according to animal nutritional requirements.

2. A comprehensive, long-term toxicological study of this water concentrate was run on mice. Nine hundred mice were included in different studies which were extended up to 150 days. In these studies, observations were made on the rate of growth, food intake, fertility, mutagenicity and mortality, blood physiology and biochemistry, liver function test behavior, and histopathology. There were very few differences between the experimental groups and the control animals. None of these changes could be related to a clear pathological syndrome.

3. A mutagenicity test was performed on male and female mice (dominant lethal mutation test). One experiment showed mutagenic properties of the water; the second was negative. Since a positive result was found in testing different concentrated water in a previous study, these results should be reviewed carefully.

4. Carcinogenicity and mutagenicity assays were carried out in tissue cultures. The concentrated water was shown to be mutagenic in hamster cells (V79). Human lung cells (WI38) were transformed by treatment with the concentrated recycled water. They gained the ability to grow on soft agar (anchorage independence) which is highly correlative with the potential of malignancy. In studies run with bacterial systems (salmonella/microsome assay), the number of revertants did not increase.

5. In vitro toxicity tests were done with human cells (WI38) using cell protein as the biological indicator. Such a test is a potential candidate to serve as a biological monitoring system in the application of reused water. Further studies are needed before conclusions are drawn.

6. The present study shows that exposure of mice to TOC levels which are 100-1000 times the present levels of our water sources did not cause significant changes in a large number of physiological and biochemical parameters. On the other hand, mutagenicity and carcinogenicity of the concentrated water were found in tissue culture assays.

SECTION 3

EXPERIMENTAL PROCEDURES

PREPARATION OF THE WATER SAMPLES

Effluent from the Blue Plains (Washington, D.C.) pilot wastewater treatment plant was concentrated in the field. A volume of approximately 400,000 liters was concentrated to approximately 800 liters over a period of about two months (October - November 1976). The 800 liters was further concentrated in the laboratory. The initial concentration procedure was based on reverse osmosis technology. A flow schematic of the reverse osmosis system is shown in Figures 1 and 2. The system, which was housed in a mobile trailer, incorporated three somewhat repetitive stages. Figure 1 shows Stages 1 and 2; Figure 2 represents Stage 3. Each stage included a set of drums, each 208 liter capacity (55 gal); high pressure pumps; reverse osmosis modules, and a back pressure valve. Stage 3 included a deionization circuit in addition to these components. Stage 1 incorporated acid addition to adjust system pH to 5.5 for optimum operation of the cellulose acetate membrane system. Stage 3 had both acid and base addition capabilities via the cation and anion exchangers. Relay-logic circuitry controlled all stages so that the system ran automatically.

Stage 1

Plant effluent or source water was pumped into Stage 1 via a sump pump until drum 1a or 1b was filled. When the drum (drum 1a, for example) was filled, the reverse osmosis system started processing the water in that drum. No more fresh water entered the drum until it had finished reverse osmosis processing. Meanwhile, the fill water had filled the other drum of the pair (1b) and was waiting for completion of the processing of drum 1a. When drum 1a had finished processing, the reverse osmosis system started operating on drum 1b while drum 1a filled for a second time. This process was repeated as many times as necessary to complete the total concentration.

Stage 1 was a single-pass system which split the process stream into two parts. The fraction retained by the membrane was more concentrated than the original feed water and was passed on to Stage 2. The water permeating the membrane was run to sewer. A concentration of approximately 1.5-fold was achieved in this stage.

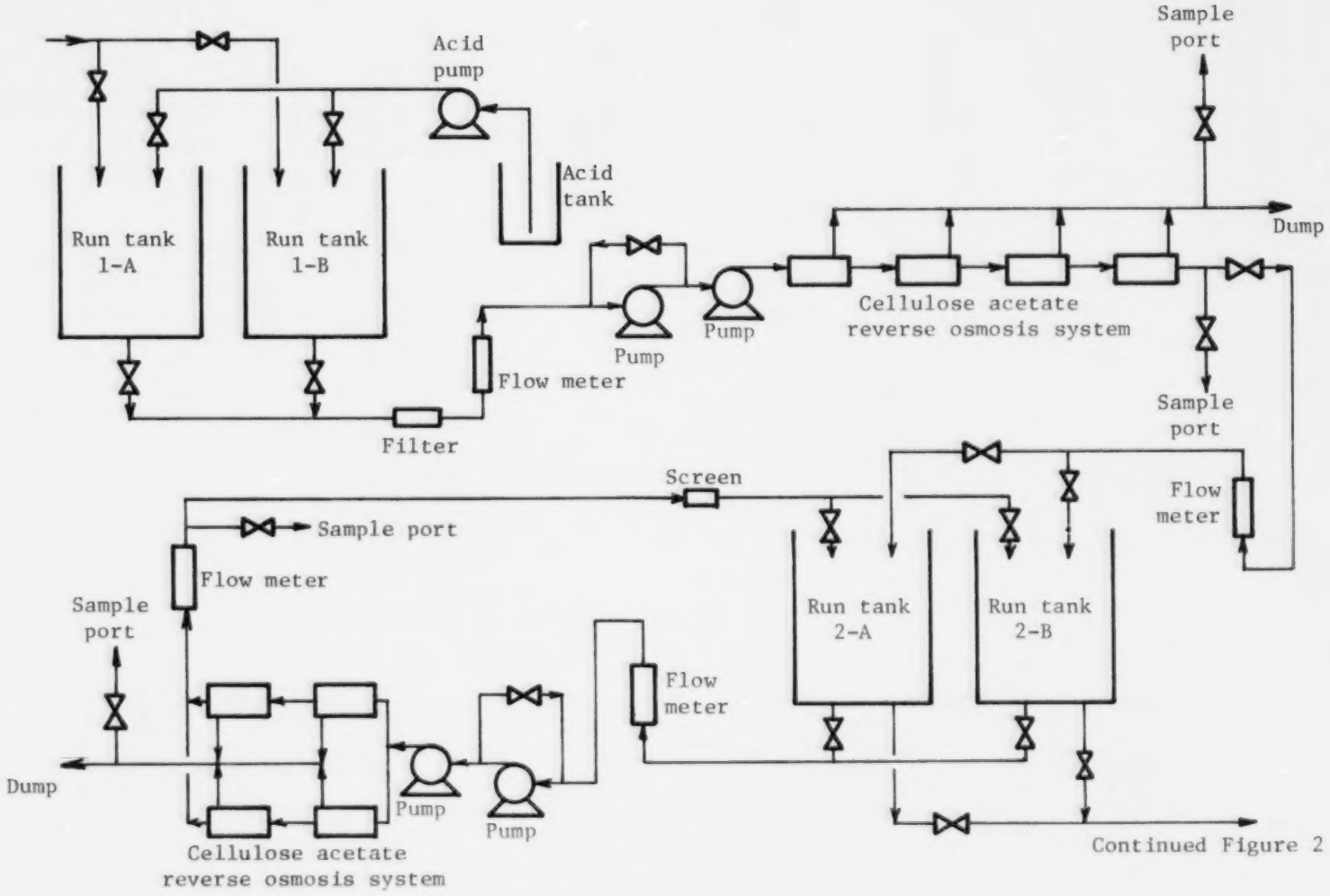


Figure 1. Stages 1 and 2 of reverse osmosis concentration system.

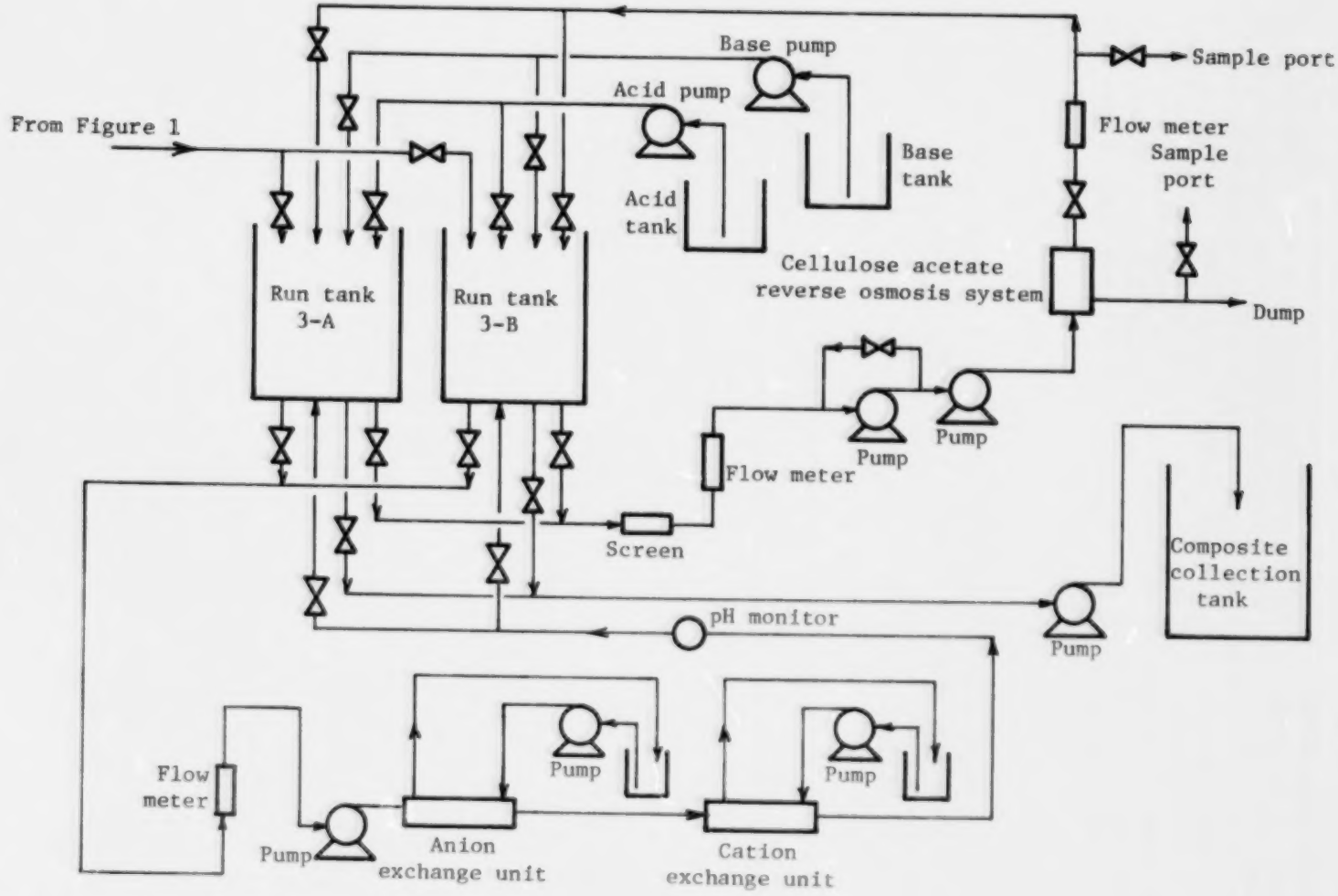


Figure 2. Stage 3 of reverse osmosis concentration system.

Stage 2

The process drums of Stage 2 were filled alternately as they were processed, as in Stage 1. The sequence was repeated as many times as necessary. All valves and pumps were controlled by relay logic. Stage 2 was a recirculating system in which the concentrated stream from the reverse osmosis modules was returned to the process drum. In this manner, a 10-fold concentration could be achieved without exceeding a 50 percent recovery (2-fold concentration) within the cellulose acetate module at any time. When the desired concentration had been reached, the concentrate in the process drum was transferred from Stage 2 into Stage 3 and the next batch for Stage 2 was started.

Stage 3

When the filling of a Stage 3 process drum was complete (after several batches from Stage 2), Stage 3 processing started. While Stage 3 processed one drum the other was filling, as in Stages 1 and 2. In Stage 3 processing, reverse osmosis concentration and ion exchange deionization (Donnan system) circuits were run concurrently.

The deionization process had two objectives: to prevent inorganic salt precipitation and to reduce the inorganic burden of the feed samples. The deionization circuit consisted of a cation exchange membrane and an anion exchange membrane in series. The concentrate from the process drum circulated past the anion exchanger, where anions were exchanged for OH^-, and then past a cation exchanger, where cations were exchanged for H^+. It was anticipated that the mass transfer of the two exchangers could be proportioned so that inorganic ion could in effect be exchanged for water (HOH).

The major problem in preparing the sample after the completion of field work was reducing the inorganic salt burden in the concentrated sample while maintaining the organic level. Several variations of the Donnan system were evaluated, but exchange rates from the anion system were not sufficient, and the actual process rate threatened to produce intolerable delays. Electrodialysis and closed-loop dialysis against deionized water were considered as alternate procedures for sample deionization. Although both methods removed significant amounts of the salt, the closed-loop dialysis was chosen since it offered maximum retention of organics and because process-size dialysis equipment was available.

The toxicological sample was prepared using the closed-loop dialysis method for overall inorganic reduction, as well as additional reverse osmosis concentration; the cation exchange system was used occasionally to balance cation levels. In addition, precipitative techniques were used to reduce levels of SO_4^{2-} and Ca^{2+} which were not easily handled by the membrane systems. Figure 3 shows the sequence in which these processes were applied to the sample.

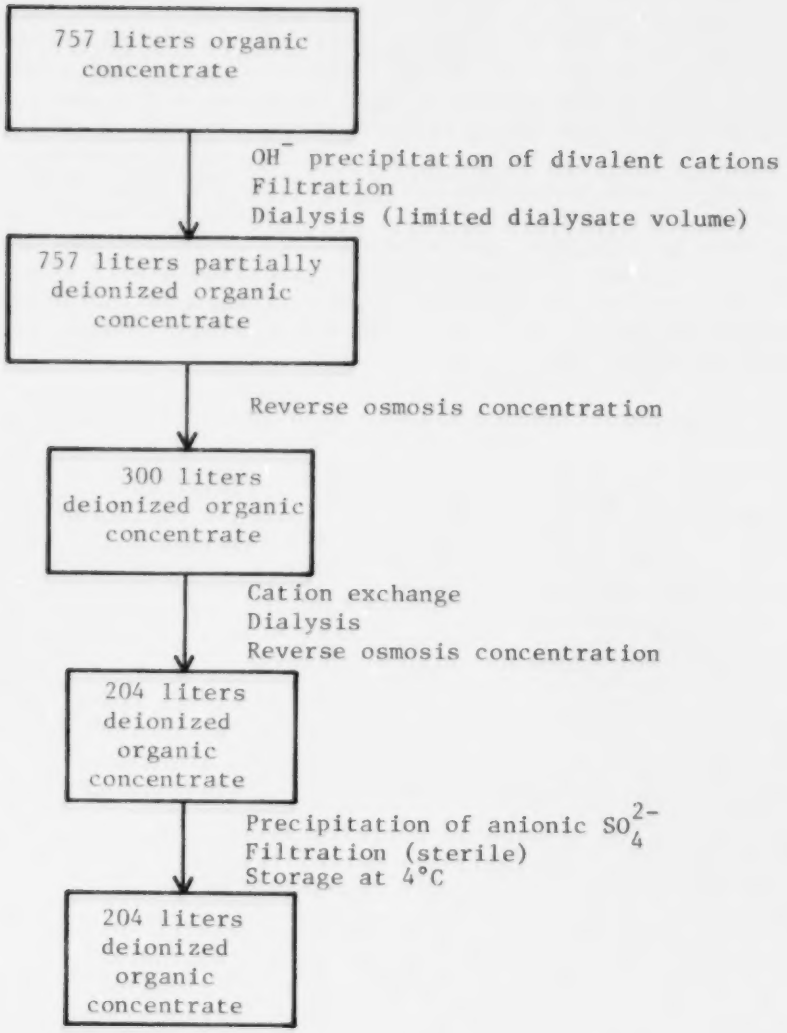


Figure 3. Effective processes used to isolate toxicological feed sample.

Hydroxide Precipitation of Divalent Cations

In early attempts to use Donnan dialysis to desalt the concentrate, there were indications that membrane fouling was occurring due to the formation of precipitates on the anion exchange membranes. These precipitates were expecially bad in stagnant areas of the exchanger; portions of the solids were soluble at low pH. These observations, coupled with the fact that pumping was against a NaOH solution in the dialyzer, indicated that the solids might be hydroxide precipitates of divalent cations (e.g., Fe, Al, Ca, Mg). At this point, the concentrate was adjusted to pH 14 with potassium hydroxide, and the alkali-insoluble precipitates were filtered out. The pH adjustment prevented formation of more of such precipitates and removed the possible source of fouling. This adjustment did not substantially improve the Donnan dialysis process but did remove some of the cationic burden in the sample. Analytical data indicated nearly an 80 percent removal of those cations contributing to the total hardness (Ca, Mg, etc.).

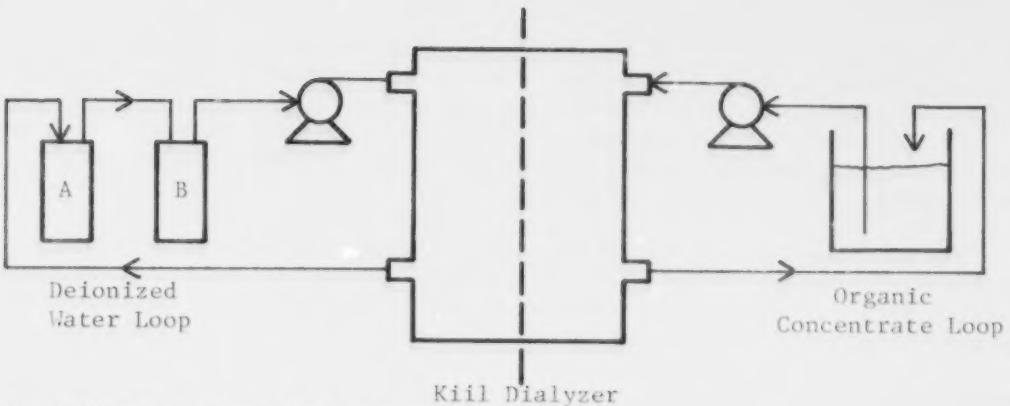
Closed-Loop Dialysis

Closed-loop dialysis removed most of the inorganics from the samples. The equipment setup shown in Figure 4 was used to effect the deionization. The Kiil dialyzer is shown in schematic form only and the drawing does not reveal the actual membrane configuration within the dialyzer. Prewashed low-flux cellulose membrane was used in the dialyzer. The membrane area of the dialyzer was approximately 0.5 m^2. With this system, 190 liters (50 gallons) of concentrate could be deionized sufficiently in about three days to allow further concentration. System operation ensured optimum retention of organics and prevented sample contaminantion. Solution flow was concurrent, with a positive differential membrane pressure of 0.34 atm (5 psi) on the concentrate loop. This, coupled with the osmotic flow phenomenon, resulted in a slight net flow of water into the concentrate loop.

Typical inorganic reductions with the system were greater than 80 percent. Retention or recovery was approximately 70 percent of organic material based on TOC.

Cation Exchange

Early analyses of the concentration sample indicated the sodium level was greater than acceptable for toxicological evaluation; the potassium level was much below acceptable levels. The sodium level was reduced by exchange with potassium via the system illustrated in Figure 5. The cationic Donnan dialysis system worked well for initial removal of large quantities of inorganics, but as the concentration gradient of inorganics across the membranes decreased, so did the efficiency of inorganics removal. Sodium and potassium levels were adjusted so that they would be acceptable for feeding studies after the final concentration.



A = XAD Absorption Resin Column
B = Mixed Bed Deionizer

Figure 4. Closed-loop dialysis apparatus.

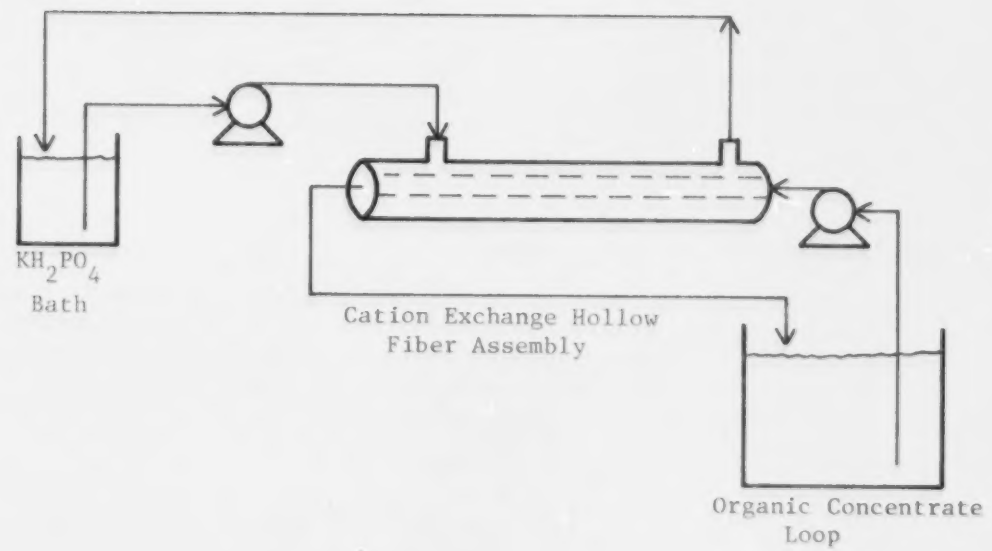


Figure 5. Cation exchange system.

Precipitation of Sulfate

Preliminary analysis of the final concentrate showed sulfate levels to be nearly twice as high as desired. The preferred method for correcting sulfate levels is use of an anion exchange system, exchanging the SO_4^{2-} for an anion deficient in the organic concentrate. However, attempts with the in-house anion exchange system, using PO_4^{3-} as an exchange anion, were unsuccessful.

The SO_4^{2-} was removed by taking advantage of the extremely limited solubility of $BaSO_4$. It was calculated from solubility data that if the level of SO_4^{2-} were reduced to 2000 mg/liter by the addition of barium, then the maximum equilibrium concentration of barium allowable in solution would be 7×10^{-4} mg/liter. Since this level was far below that of any anticipated toxicological problem level, the precipitation was carried out. The barium was added in the form of a mixture $Ba(Cl)_2 \cdot 2H_2O$ and $Ba(OH)_2 \cdot 8H_2O$. The total amount of barium added was calculated to be equal to that necessary to reduce the SO_4^{2-} to 2000 mg/liter. The dichloride salt was chosen for its solubility and its lack of effect on solution pH. Only a limited amount of barium could be added as the chloride; the balance of the required barium was added as the hydroxide. This necessitated pH readjustment with H_3PO_4. The $BaSO_4$ precipitate was filtered from the organic concentrate mixture by sterile filtration.

Sterile Filtration

The final filtration of the concentrated water required a sterile product for storage. The solution to be filtered contained $BaSO_4$ in substantial quantities and was moderately turbid. The $BaSO_4$ solids rapidly settled to a 3/8-inch layer on the bottom of a 210-liter (55-gallon) drum. The filter setup shown in Figure 6 was used to filter the solids and sterile filter in one pass. The product was a clear, yellow-brown solution, collected in sterilized one-gallon glass bottles. These bottles were kept at 4°C until used.

FOOD PREPARATION

The diet to be fed to the mice in the toxicological experiments was composed of 50 percent solids and 50 percent water. The studies included five groups: A-control, B-water sample diluted 1:8 with deionized water, C-diluted 1:4, D-diluted 1:2, and E-undiluted concentrated water. The undiluted concentrate contained about 700 mg/liter of total organic carbon (TOC).

The solid portion of the mixture consisted of three parts: (1) a basal mixture of the main nutritional ingredients (Table 1), (2) the vitamin mixture (Table 2), and (3) a salt mixture (Table 3). Mixtures (1) and (2) were purchased from Teklad, Madison, Wisconsin. Wesson's modification of Osborne-Mendel mix (8) was followed in preparing the salt mixture. The composition of the total diet was:

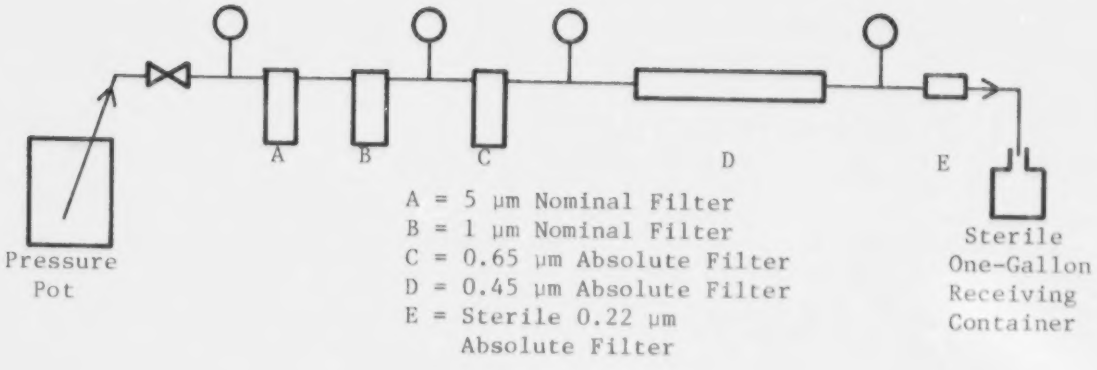


Figure 6. Filter assembly for final solution concentration.

TABLE 1. BASAL MIXTURE FOR THE TOXICOLOGICAL DIET

	(g/kg)
Casein Vitamin Free Test	245.5
Sucrose	158.5
Dextrose, Hydrate, Technical	214.3
Dextrin, White, Technical	214.3
Corn Oil	167.4
	1000.0

TABLE 2. COMPOSITION OF VITAMIN MIXTURE

	(g/kg)
P-Aminobenzoic Acid	0.1
Ascorbic Acid	0.2
Biotin	0.0005
Vitamin B-12 (0.1% trituation in mannitol)	0.05
Calcium Pantothenate	0.05
Choline Dihydrogen Citrate	0.7515
Folic Acid	0.002
Inositol	0.2
Niacinamide	0.05
Pyridoxine HCl	0.01
RiRiboflavin	0.02
Thiamine HCl	0.01
Dry Vitamin A-D (500,000 units of Vitamin A Acetate/g and 50,000 units of Vitamin D-2/g)	0.006
Dry Vitamin E Acetate (500 U/g)	0.2
Menadione	0.005

TABLE 3. COMPOSITION OF SALT MIXTURE

	(percent)
Calcium carbonate, $CaCO_3$	21.00
Calcium Phosphate Tribasic, $Ca_3(PO_4)_2$	14.90
Cupric Sulfate, $SuSO_4 \cdot 5H_2O$	0.04
Ferric Pyro Phosphate	1.47
Magnesium Sulfate, $MgSo_4$	9.00
Manganese Sulfate, $MnSO_4 \cdot H_2O$	0.02
Potassium Aluminum Sulfate, $K_2Al_2(SO_4)_4 \cdot 24H_2O$	0.01
Potassium Chloride, KCl	12.00
Potassium Iodide, Kl	0.01
Potassium Phosphate, Monobasic KH_2PO_4	31.00
Sodium Chloride, NaCl	10.50
Sodium Fluoride, NaF	0.06

TABLE 4. IONS REQUIRED IN TOXICOLOGICAL DIET
(mg/2kg food)

Cation	Amount	Anion	Amount
Ca	5,677	Co_3	5,040
Fe	218	SO_4	2,900
Mn	2.9	PO_4	12,772
Mg	720	Cl	4,834
K	6,075	I	1.5
Al	0.4	F	10.4
Na	1,663		
Cu	4.0		

	grams
Basal mixture	920
Vitamin mixture	10
Salt mixture	40
Agar	30
Water	1000

Based on the figures in Table 3, the weights of the required ions for the diet were calculated and are given in Table 4. The water analysis for inorganic constituents is given in Table 5. Calculations based on the figures in Tables 4 and 5 determine the amount of salts necessary for the different dilutions of the concentrated water in the different groups (Tables 6 and 7).

The diet was prepared by mixing an agar solution with the solids to make a gelatinous food. One liter of water was heated to 70°C, and 30 g of agar was slowly added while stirring until the agar was completely dissolved. The basal mixture and the appropriate salt mixture were placed in a mechanical stirrer and the agar solution was added and mixed thoroughly until the temperature reached 45°C. The vitamin mix was added to this blend and stirring was continued for another 10-15 minutes. This mixture was poured into plastic jars and stored at 5°C. The diet was prepared each week and food was replaced daily in the animals' cages.

TOXICOLOGICAL TESTS

In Vivo Studies

All the studies were carried out on mice-strain B6C3F1 purchased from Charles River. Table 8 summarizes the different in vivo studies that were included in the project and Table 9 gives the various experiments and tests that were carried out. Table 10 provides a summary of the number of mice used for each study. In Study P, mice aged 10 weeks were split into cages; two females and one male in each cage. In Study I, mice aged 8 weeks were each placed in a separate cage. In these two studies, the mice were quarantined for two weeks before the experiment started. Mice of Studies II and III were born to the mice in Study P and were exposed throughout gestation and lactation before the experiment started (immediately after weaning). Study IV was exactly the same as Study I with the addition of a 90-day recovery period. The mice in Studies P, I, II, III, and IV were at the ages of 3, 5, 4, 6 and 6 months, respectively, when the studies ended. Mice from Study P were checked for gross clinical or behavioral changes, food consumption, body weights and lethality. These animals were also used to produce the animals in Studies II and III. The first dominant lethal mutation test and the first reproduction experiment test were done on the animals of Study P.

TABLE 5. CHEMICAL ANALYSIS OF THE CONCENTRATE

Parameter	mg/l	Parameter	mg/l
As	<0.01	Na	1300
Ba	<0.5	K	4300
Cu	<0.10	Ca	632
Al	0.40	Mg	15
Cr	<0.01	Cl	4400
Zn	2.00	SO_4	2650
Pb	<0.01	PO_4	1500
Ag	<0.01	I	2.9
Hg	<0.01	Total Hardness (as $CaCO_3$)	1640
Se	<0.01		
Mn	0.17	Mg Hardness (as $CaCO_3$)	60
Fe	1.70		
Co	<0.20	Total Alkalinity	270
B	<15	Total Dissolved Solids	132,000
Si	1.3		
NO_3-N	<0.1	pH	5.5
NO_3-N	<0.01	Stability index	7.3
F	2.6	Saturating index	0.9
		Odor Threshold	4
		Turbidity	3.2
		Color PCU	260
		Total Organic Carbon	704

TABLE 6. AMOUNTS OF IONS ADDED TO TOXICOLOGICAL DIETS
(mg/kg)

Ion	Undiluted	1:2	1:4	1:8
Ca	5045	5361	5522	5597
Fe	218	218	218	218
Mn	2.7	2.7	2.7	2.7
K	700	700	700	700
Al	0.0	0.2	0.3	0.4
Na	363	1013	1338	1500
CO_3	5126	5275	5275	5275
SO_4	250	1575	2240	2570
PO_4	11,460	12,022	12,417	12,590
Cl_4	400	2634	3734	4284
I	1.0	1.2	1.5	1.5
F	8.0	9.0	10.0	10.5

TABLE 7. AMOUNTS OF SALTS ADDED TO TOXICOLOGICAL DIETS
(mg/kg)

Salt	Undiluted	1:2	1:4	1:5	Control
$CaCO_3$	5666	6000	6000	6000	8400
$CaHPO_4$	9482	9482	9482	9482	5960
$FePO_4$	600	600	600	600	600
KH_2PO_4	6300	7000	7500	8000	12,400
$MgCl_2$	535	535	535	535	--
$MgSO_4$	312	312	312	312	3600
$MgCO_3$	1750	1750	1750	1750	--
$NaCO_3$	841	1000	1200	1400	--
KCl	--	5533	7844	9000	4800
Na_2SO_4	--	2329	3313	3800	--
NaF	17	19	21	23	22.8
KI	1.5	1.7	2.0	2.0	2.0
$KAlSO_4$	--	3.6	3.6	3.6	3.6
$CuSO_4$	15.6	15.6	15.6	15.6	15.6
$MnSO_4$	8.0	8.0	8.0	8.0	8.0
$NaCl_4$	--	--	--	--	4200.0

TABLE 8. SUMMARY OF STUDIES PERFORMED

Study	Exposure Time	Number of Animals	
		Males	Females
P	14 days	50	100
I	90 days	200	200
II	Gestation, Lactation and another 90 days	100	100
III	Gestation, Lactation and another 150 days	50	50
IV	90 days and another 90 days on regular diet	50	-

In addition to the above-mentioned tests (in Study P), mice from Studies I, II, and III were tested for hematology, blood chemistry, mixed function oxidase activity, motor activity, and pathology. Another dominant lethal mutation experiment was run on females and males from Study I, and a second reproduction assay on Study III. Body weights were measured twice a week. General observation and food consumption measurements were made daily.

Hematology--

Mice were bled from their tails (after warming) and a blood sample was taken with the aid of microcapillaries. Hemoglobin was measured by Coulter Hemoglobinometer Model HGBR Serial No. 2090 which is an automatic rinsing hemoglobinometer reading the color intensity of cyanomethemoglobin. Red and white blood cells were counted with a Coulter Model FN No. 7356 based on nonoptic measurement, one-by-one counting, and sizing particles suspended in solution. The tests were run after 70 days of exposure, and animals for the tests were chosen randomly using the table of random digits.

Blood Chemistry--

Each day approximately 50 animals from each group were tested. Animals were fasted overnight before sacrifice. Blood was drawn by heart puncture under anesthesia and was let stand for about 30 minutes at room temperature. After this period, the blood was centrifuged in microtubes containing a silicon material which after centrifugation seeks the interface between the clot and the serum and forms a barrier between the two phases ("Microtainer", Becton-Dickinson, New Jersey). The tubes were stored at 5°C and tested after 24 hours. Serum was diluted with distilled water (1:1). This dilution had no effect on the results. The samples were tested in a computer-controlled Autoanalyzer (SMAC, Technicon, Tarrytown, New York). All daily samples were run together at the same time, and a reference control was run after every three samples. The following parameters were tested:

TABLE 9. TOXICOLOGICAL TESTS (IN VIVO)

General Physiology	Blood Chemistry
Food Consumption	Glucose
Body Weight	Chloresterol
Hematology	Triglyceride
Hemoglobin	Total Protein
Red Blood Cell Count	Albumin
White Blood Cell Count	Calcium
Mixed Function Oxidase Activity	Phosphorus
Motor Activity	Sodium
Reproduction	Potassium
Dominant Lethal Mutation	Chloride
Pathology	Carbon Dioxide
Heart	Urea Nitrogen
Lungs	Uric Acid
Spleen	Total Bilirubin
Liver	Creatinine
Kidney	Alkaline Phosphate
Adrenals	LDH
Brain	GOT
Tests	GPT
Ovaries	CPK
Microscopy and Tissue Weights	

TABLE 10. NUMBER OF ANIMALS IN THE DIFFERENT STUDIES

	Group A		Group B		Group C		Group D		Group E	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Study P	10	20	10	20	10	20	10	20	10	20
Study I	40	41	41	41	42	39	42	39	39	39
Study II	19	19	21	20	19	19	19	21	16	23
Study III	10	10	10	10	10	10	10	10	9	10
Study IV	9	-	9	-	-	-	10	-	10	-

Glucose (Glu)	Carbon dioxide (CO_2)
Cholesterol (Chol)	Urea nitrogen (UN)
Triglycerides (Trig)	Uric acid (UA)
Total protein (TP)	Total bilirubin (Bili)
Albumin (Alb)	Creatinine (Creat)
Calcium (Ca)	Alkaline phosphatase (AP)
Inorganic phosphorus (P)	Lactate dehydrogenase (LDH)
Sodium (Na)	Glutamic pyruvic transaminase (GPT)
Potassium (K)	Glutamic oxaloacetic transaminase (GOT)
Chloride (Cl)	Creatine phosphokinase (CPK)

Glucose--This assay is based on the glucose oxidase-peroxidase procedure. The specificity of glucose oxidase is combined with a peroxidase indicator couple [3-methyl-2-benzothiazolinone hydrazone (MBTH) and N,N-dimethylaniline (DMA)] to form a stable, intensely colored, water-soluble indamine dye, which was read at 600 nm.

$$\text{Glucose} + O_2 + H_2O \xrightarrow[\text{Oxidase}]{\text{Glucose}} \text{Gluconic acid} + H_2O_2 \quad (1)$$

$$\text{MBTH} + \text{DMA} + H_2O_2 \xrightarrow{\text{Peroxidases}} \text{Indamine Dye} + H_2O + OH \quad (2)$$

Cholesterol--The following mechanisms have been proposed for the reaction of cholesterol with sulfuric acid.

$$\text{Cholesterol} + H_2SO_4 \longrightarrow \text{bis-cholestadienyl monosulfonic acid} \quad (1)$$

$$\text{bis-cholestadienyl monosulfonic acid} + H_2SO_4 \longrightarrow \text{bis-cholestadienyl disulfonic acid} \quad (2)$$

The sample was added to a chilled (0°C) color reagent and the mixture allowed to reach room temperature. The reaction is highly exothermic; therefore, special care was taken to keep the optimum reaction temperature. The absorbance was measured at 630 nm.

Triglycerides--Triglycerides are specifically hydrolyzed by lipase to glycerol and free fatty acids. The glycerol product is phosphorylated to glycerol phosphates by glycerol kinase which is then coupled to pyruvate kinase to form pyruvate. This product enters the well-known NADH-NAD reaction catalyzed by LDH. The whole reaction was followed by measuring the absorbance at 340 nm. A control was subtracted from the test value to correct for changes in the absorbance caused by endogenous serum interferences.

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Free fatty acids} \quad (1)$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[\text{Kinase}]{\text{Glycerol}} \text{Glycerol phosphate} + \text{ADP} \quad (2)$$

$$\text{ADP} + \text{Phosphoenolpyruvate} \xrightarrow[\text{Kinase}]{\text{Pyruvate}} \text{ATP} + \text{Pyruvate} \quad (3)$$

$$\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}$$

Total Protein--Protein was determined by the biuret method. The protein combines with the copper in the biuret reagent to form a purple complex which was read at 550 nm.

Albumin--Bromocresol green (BCG) combines specifically with albumin to form a stable complex. The albumin-BCG complex was read directly at 630 nm. A special reagent was added to the reaction mixture to minimize the absorbance of the reaction blank to prevent turbidity and to provide linearity.

Calcium--The calcium method uses the metal complexing dye "Cresolphthalein Complexone" which binds calcium ions in alkaline medium. The product of this intereaction is a pink calcium dye complex with a maximum absorption at 570 nm. First, serum was added to a diluted solution of HCl containing 8-hydroxyquine which binds the free magnesium ions present in the serum. A sample was mixed with Cresolphthalein Complexone containing 8-hydroxyquininoline. Upon the addition of diethylamine, a color complex is formed between the calcium and the dye. The absorbance of the reaction product was measured at 570 nm.

Inorganic phosphorus--The serum phosphorus is reacted with ammonium molybdate. Instead of the popular method to reduce the complex phosphomolybdate with reducing agent, this assay is based on the fact that the unreduced complex absorbs ultraviolet light. The absorbance was measured at 340 nm.

Sodium--The sodium method is a direct potentiometric procedure for the quantitative measurement of sodium in serum by use of a sodium-selective glass electrode. The sodium selective electrode responds to sodium ions according to the Nernst equation.

Chloride--This is a colorimetric procedure:

$$Hg(SCN)_2 + 2Cl^- \longrightarrow HgCl_2 + 2(SCN)^- \quad (1)$$

$$3(SCN)^- + Fe^{+3} \longrightarrow Fe(SCN)_3 \quad (2)$$

The absorbance of the red complex $Fe(SCN)_3$ was measured at 480 nm.

Carbon dioxide--Carbon dioxide is present in the serum also as H_2CO_3 and HCO_3. CO_2 and H_2CO_3 are present in serum in relatively small amounts. All the species were in equilibrium with each other. The method used to measure CO_2 was based on the release of CO_2 by an acid. The released CO_2 is absorbed by an alkaline solution containing phenolphthalein, causing a change in pH which results in a decrease in the abosrbance at 550 mμ.

Potassium--The potassium method, like the sodium method, is a direct potentiometric procedure by means of a potassium ion selective electrode.

Urea nitrogen--In a weak acid solution, diacetyl monoxime is hydrolyzed to diacetyl which, in turn, reacts directly with urea in the presence of acidic ferric ions. The presence of thiosemicarbazide intensifies the color of the reaction. The absorbance was read at 520 nm.

Total bilirubin--The sample reacts with "Diazo" reagent to form azobilirubin complex. To this mixture a strong alkaline sodium potassium tartarate buffer was added which solubilizes protein and eliminates the effect of variation in sample pH. The colored complex was measured at 600 nm.

Creatinine--The creatinine method is based on the reaction of saturated picric acid with creatinine in an alkaline medium to form a red color chromogram which was measured at 505 nm.

Alkaline phosphatase--The method is based on the hydrolysis of p-nitrophenyl phosphate. The product at alkaline pH gives a yellowish color.

$$\text{P-nitrophenyl phosphate} \xrightarrow[Mg^{2+};\ 37°C;\ pH\ 10.25]{\text{Alkaline Phosphataes}} \text{P-nitrophenol} + H_3PO_4 + H_2O$$

The absorbance was read at 410 nm.

Lactate dehydrogenase--This enzyme catalyzes the following reaction. 1-Lactic acid + $NAD^+ \rightleftarrows$ Pyruvic acid + NADH + H^+. NADH has an absorption peak at 340 nm and the enzymatic activity is porportional to the amount of NADH produced during a fixed time interval. Under specific conditions of pH, temperature and substrate concentrations, the reaction obeys zero time kinetics.

Glutamic oxaloacetic transaminase--The enzymatic reaction of GOT:

$$\text{Aspartate} + \alpha\text{-Ketoglutarate} \longrightarrow \text{oxaloactate} + \text{glutamate} \qquad (1)$$

is coupled to malic dehydrogenase.

$$\text{Oxaloacetate} + \text{NADH} \xrightarrow{MDH} \text{NAD} + \text{Malate}. \qquad (2)$$

All the reagents except ketoglutarate were mixed with the serum sample and preincubated, followed by the addition of α-ketoglutarate which starts the reaction, which was followed at 340 nm.

Glutamic pyruvic transaminase--The enzymatic reactions are as follows:

$$\text{Alanine} + \alpha\text{-ketoglutarate} \xrightarrow{GPT} \text{pyruvate} + \text{glutamate} \qquad (1)$$
$$\text{Pyruvate} + \text{NADH} \xrightarrow{LDH} + \text{Lactate} + \text{NAD}$$

Again, all the reagents were incubated except ketoglutarate, which is added after the preincubation. The reaction is started by adding α-ketoglutarate.

Creatinine phosphokinase (CPK)--The enzymatic reaction is as follows:

$$\text{Creatine phosphate} + \text{ADP} \xrightleftharpoons{\text{CPK}} \text{Creatine} + \text{ATP}$$

Cystain was added to ensure maximal activity. The reaction is stopped by N-ethylmaleimide which also prevents the sulfhydryl groups from interfering with the creatine coupling reaction. Diacetyl/orcinol reagent was added and a condensation product was formed which developed a strong color upon the addition of the sodium hydroxide solution and incubation at 45°C. The presence of EDTA in the reagents prevents the precipitation of $Mg(OH)_2$. The color was measured at 520 nm.

Statistical Analyses--Statistical analyses were performed on a PDP-10 computer, using SPSS (Statistical Package for the Social Sciences). The condescriptive procedure and the T-test subprogram were used (9). All groups were compared statistically to the control group (A).

Liver Mixed Function Oxidase (MFO) Activity--

Sleeping times induced by sodium hexabarbital (100 mg/kg body weight) were measured as a function of MFO activity. Latent time is defined as the time between injection and the loss of righting reflex, and sleeping time is the time between loss and gain of righting reflex.

Motor Activity Measurement--

This measurement was taken in a hexagonal box equipped with 2 perpendicular light beams and 2 photoelectric cells connected to 2 independent counters. Each animal was put in the box for 10 minutes and counts were recorded after 5 and 10 minutes. The counts from the 2 counters were combined at each time. All tests were run at the same time of the day in a dimly lighted room. Animals were selected for the test by using the table of random digits. Animals selected for the behavior test were excluded from the mixed function oxidase test and vice versa.

Dominant Lethal Mutation Test--

Each selected male was mated with two unexposed females for 5 consecutive weeks after ceasing the exposure. Each week, 2 new females were put in the cage with the male and the mated females were left for another week, and then sacrificed. The number of live and dead fetuses was counted. The results were tested by nonparametric statistics (Mann-Whitney U test) (10, 11). Data from all the experimental groups were combined for analysis since the results did not differ significantly from group to group.

Pathology--

A board-certified or board-eligible veterinary pathologist with experience in laboratory animal pathology was responsible for all pathology procedures, evaluations and reporting. Well-qualified laboratory technicians performed post-mortem activities and slide preparations.

All animals from this study were given a complete post-mortem examination. The tissues and organs listed in Table 11 were examined *in situ*, then removed and incised properly to ensure adequate fixation and placed in 10% neutral buffered formalin. Following adequate fixation, the tissues listed in Table 12 were trimmed and embedded in paraffin blocks sectioned according to standard histological procedures and stained by the hematoxylin and eosin method.

In Vitro Studies

Salmonella/Microsome Test--

The procedure followed that of Ames, *et al*. (12) Tests were run on strains TA1535, 1537, 1538, 98, and 100. Assays were performed with and without activation because some compounds do not require conversion to active forms by mixed function oxidases.

The activation system consisted of microsomal fractions prepared from adult male Sprague-Dawley rats (150-200 g) injected with 500 mg/kg Aroclor 1254 five days prior to sacrifice. All food was removed 24 hours before microsome preparation. The livers were excised, washed, weighed, and homogenized in a Teflon homogenizer with three volumes of 0.15M KCl. Care was taken throughout the procedure to maintain the tissue at 4°C. The postmitochondrial supernatant (S9 fraction) was prepared by centrifugation at 9000 x g for 10 minutes. The supernatant was frozen and stored at -80°C in 1 ml aliquots.

The water sample was added directly to the molten top agar and poured onto the plate together with the indicator test organism with or without activation system. Negative controls included plates to measure the number of spontaneous colonies, plates to check for the sterility of the microsomes, and the water.

Mutagenicity Tests with Mammalian Cells--

Mutagenesis testing was performed according to the method described by Kuroki, *et al*. (13) with slight modifications. V79 cells, derived from male Chinese hamster lungs, were used for all mutagenesis tests. Cells were cultured in Dulbecco's modified minimum essential medium (DMEM) supplemented with 10% heat-inactivated (56°C, 30 min) fetal bovine serum (IFBS). For subculture, cultures were trypsinized with 0.05 percent trypsin in 0.02 percent EDTA. All cultures were incubated at 37°C in a water-saturated, 10 percent CO_2 atmosphere.

For mutagenesis testing, V79 cells were plated in 100-mm petri dishes (Falcon) at a concentration of 6×10^5/plate and were incubated overnight. The cells were then incubated in 2 ml of the reaction mixture consisting of 0.3 ml S9 fraction (approximately 9 mg protein), 0.3 ml Sorensen-phosphate buffer (0.055M, pH 7.4, containing 0.9 percent NaCl and 0.49 mg $MgCl_2 \cdot 6H_2O$ per ml), 0.3 ml glucose-6-phosphate solution (13 mg/ml in PBS), 0.1 ml NADP solution (6.3 mg/ml in PBS) per ml, and 0.2 ml PBS. The final concentrations in the reaction mixture were 20 µmoles of inorganic phosphate, 1.53 µmoles G-6-P, and 0.8 µmoles NADP.

TABLE 11. TISSUES AND ORGANS EXAMINED IN GROSS NECROPSY

Tissue masses or suspect tumors	Thigh muscle
Regional lymph nodes	Sciatic nerve
Skin	Sternebrae, vertebrae; or femur (plus marrow)
Mandibular lymph node	Costochondral junction, rib
Mammary gland	Thymus
Salivary gland	Gall bladder
Larynx	Pancreas
Trachea	Spleen
Lungs and bronchi	Kidneys
Heart	Adrenals
Thyroids	Bladder
Parathyroids	Seminal vesicles
Esophagus	Prostate
Stomach	Testes
Duodenum	Ovaries
Jejunum	Uterus
Ileum	Nasal Cavity
Cecum	Brain
Colon	Pituitary
Rectum	Eyes
Mesenteric lymph node	Spinal cord
Liver	

TABLE 12. TISSUES FOR MICROSCOPIC EXAMINATIONS

Heart	Brain (three sections including cortex, midbrain and cerebellum)
Lungs and mainstem bronchi	Testes (males)
Kidneys	Bladder and prostate
Spleen	Ovaries (females)
Liver	Uterus (females)
Mandibular lymph node	

The concentrated water was combined 1:1 in 2x medium prior to addition to the reaction mixture. Samples were added to plates containing the reaction mixture and were incubated for 1 hr at 37°C. One µg/ml benzopyrene was used as a positive control. Other controls include untreated cells and cells exposed to the S9 mix without sample.

Following treatment, the cells were washed three times in PBS and fresh medium was added. Cells were left for 2 hours. The cells were then trypsinized, counted and replated for determination of the induced cytotoxicity and mutagenicity. To measure cytotoxicity, 100 cells from each group were plated in each of four 60-mm plates in 4 ml DMEM plus 10 percent IFBS. After 8 days, these plates were fixed in absolute methanol and stained with 10 percent Giemsa. Production of ouabain-resistant clones was measured by plating 10^5 cells in each of 16 plates per point in 4 ml DMEM plus 10 percent IFBS. Forty-eight hours after plating, ouabain (final concentration in each plate = 1 mM) was added to the mutagenesis plates. These cultures were fixed and stained as above, 14 days after plating. The frequency of ouabain-resistant colonies was calculated per 10^6 surviving cells, taking into account the cytotoxicity and the number of cells plated.

The water was also treated for mutagenecity in the absence of the activation system. V79 cells were seeded in 60-mm plates at concentration of 10^3 and 10^2 cells per plate. After 24 hours, the medium was removed and replaced by a final volume of 2 ml of medium and H_2O combined with 2X MEM (1:1). MNNG (1 µg/ml) was used as positive control. All plates were treated for 4 hours at 37°C, washed three times with PBS and 4 ml of fresh DMEM 10 percent IFBS added. Ouabain was added 48 hours after treatment to the plates containing 10^5 cells, as above. Toxicity plates were fixed and stained as above 7 days, and the mutagenicity plates were fixed and stained 14 days after treatment.

Soft Agar Transformation Assay--

For the soft agar transformation assay, $WI38_6$ stock cells were trypsinized and suspended at a concentration of 1.5×10^6 cells/ml in MEM + 10% IFBS. This assay was performed with and without the S_9 activation system described earlier. Benzo(a)pyrene and MNNG were used as positive controls. In samples containing the activation system, 1 ml of the S_9 mix was added to the 1-ml cell suspensions. The concentrated water and control compounds were then added and all samples were incubated for 2 hours at 37°C in an orbital shaker. After incubation, the cells were centrifuged at 50 x g for 10 min, and the supernatants containing the compound and microsomes were discarded. Each pellet of cells was resuspended in 15 ml of growth medium to which was added 1.25 ml of 2.5 percent agar solution. The final concentration of agar was 0.3 percent. The suspension was quickly mixed and layered, in 1.5 ml volumes, on each of ten 60 mm petri dishes containing 5 ml of MEM and 0.9 percent agar. The plates were allowed to gel and are incubated at 37°C in a CO_2 incubator.

Since there is evidence that transformation, at least in diploid cells, is not a one-step process, and may be detectable in soft agar only after several generations following treatment, replicate cultures were seeded in 75 cm^2 Falcon flasks and were maintained for subsequent assays. Approximately every 2 weeks, these cells were assayed for soft agar transformation by seeding them on agar plates as above. These cells received no additional exposure to the compounds used in the initial treatment.

Soft agar plates were monitored for at least 3 weeks for the presence of transformed colonies. Only those colonies exceeding 100 µ diameter are scored. Results were expressed as the number of transformed colonies per 10^5 survivors. Positive results must be 2.5 X untreated negative controls.

WI38 Toxicity Assay--

WI38 cells, derived from human embryonic lung, were seeded in culture tubes in 2 ml MEM plus 10 percent IFBS and placed on an orbital shaker at 37°C. After 24 hours, 3 tubes were washed 3 times with PBS, drained and were frozen to determine base protein levels at zero time. Tubes were exposed to water samples at either various concentrations or different exposure times. In dose-response experiments, the tubes were treated for 72 hours. In kinetics experiments, the cells were exposed for the specified time, washed and frozen. Controls were used for each point, including untreated controls and controls for S9 toxicity in experiments employing an activation system. The activation system consisted of Kuroki's mix described above with a reduction in final S9 concentration to 25 µl per ml. Tubes were assayed according to Lowry and were read at 660 nm. Both percent inhibition and protein values were compared. Cells were counted in diluted samples after trypsinization with the aid of hemocytometer.

SECTION 4

RESULTS

PREPARATION OF THE RAW WATER

The water used in this study was concentrated from finished water prepared in a treatment system located on the grounds of the Washington, D.C., Blue Plains wastewater treatment facility. The treatment procedures and the quality of the water obtained are described below. This information is taken from EPA reports (14,15) concerning operation of the Blue Plains pilot plant. The system degritted the municipal wastewater, using a screening device to remove coarse materials, and included processes for lime clarification, dispersed growth nitrification, denitrification, activated carbon adsorption, alum treatment, and chlorination.

A diagram of the treatment system is presented in Figure 7, and design and operating conditions are summarized in Table 13. The treatment system was operated on a continuous basis with operators assigned to three 8-hour shifts each day. The system was modular in design to permit additions and modifications without affecting system integrity.

Performance of the Water Treatment System

Virus Removal--

Animal viruses in raw sewage samples ranged from 1750-17000 PFU/100 liters. No viruses were detected in the final effluent, even after concentration. Sample volumes were in the range of 180-900 liters. A typical concentration was from 380 liters down to 10-20 ml.

Metals--

Results of about 50 tests of effluent samples, each analyzing 14 metals, showed that none of the metal concentrations exceeded the levels for drinking water cited in the EPA regulations (16). Other experiments showed that lime treatment is primarily responsible for the reduction in metal concentration.

General Organics--

The treatment system is capable of producing effluents with low levels of total organic carbon. Some 230 samples showed a TOC level of 74.1 $\pm$ 11.0 mg/liter (M $\pm$ S.D.) in the influent and only 2.79 $\pm$ 1.35 mg/liter in the effluent. The mean phenol concentration in 54 effluent samples was 3.66 $\pm$ 1.52 µg/liter, as compared to the 1962 drinking water standard of 1 µg/liter.

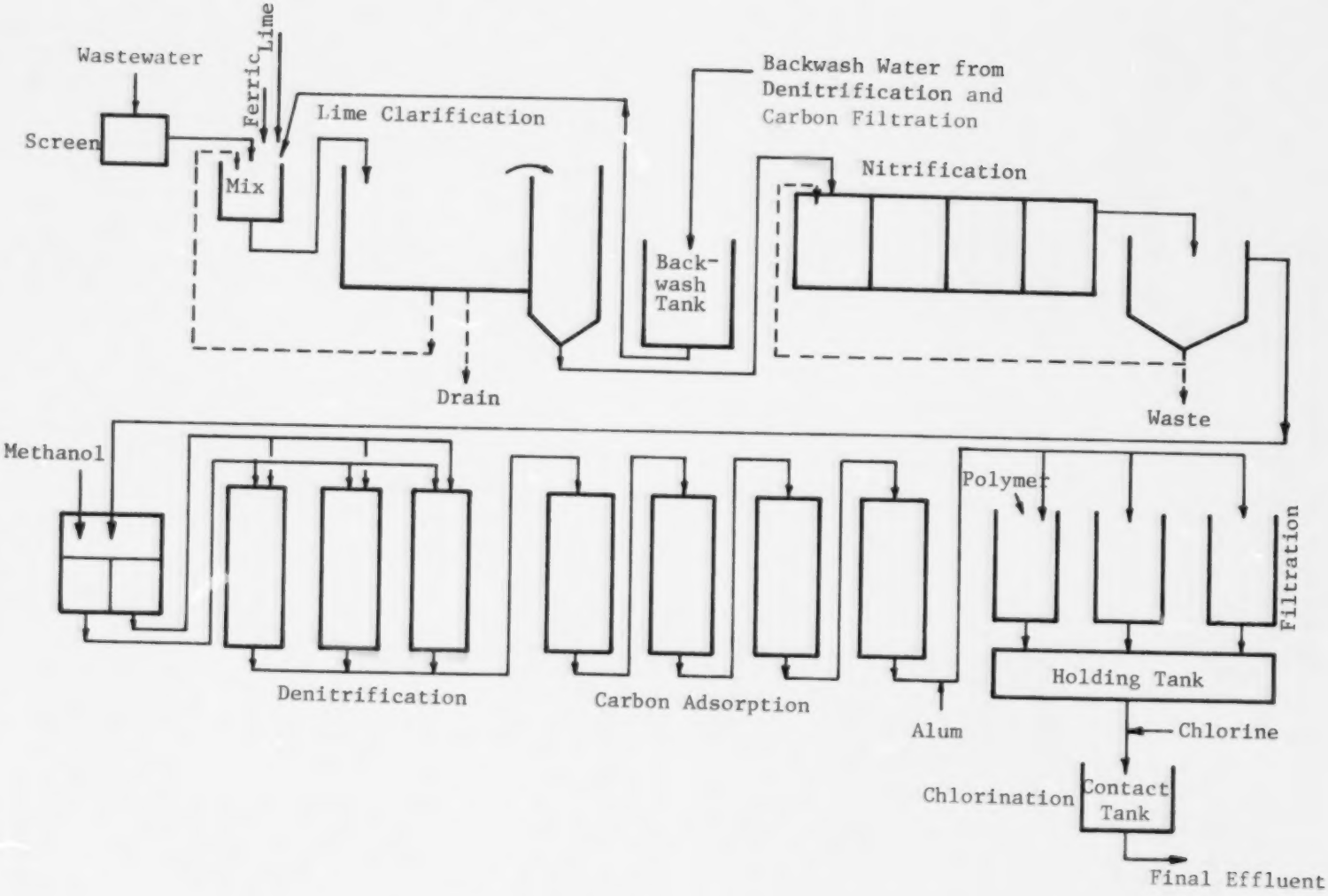


Figure 7. Blue Plains water treatment system.

TABLE 13. DESIGN DATA AND OPERATING CONDITIONS FOR BLUE PLAINS TREATMENT SYSTEM

Raw Wastewater (Constant Flow)	2.2 liters/second (35 gpm)
Screening Device	
Type	Bauer Hydrasive Model 552
Size of Openings	0.040 inch screen
Lime Clarification	
Lime Dosage (pH 10.0) (as $CaCO_3$)	200 mg/l
$FeCl_3$ Dosage (as Fe)	15 mg/l
Hydraulic Loading Rate	1050 gpd/ft^2
Detention Time	2.7 hours
Sludge Wasting Rate	2% to 3%
Percent Solids in Waste Sludge	1.5% to 2.0%
Nitrification (Suspended Growth)	
Detention Time	3.5 hours
MLSS	2000 mg/l
SRT	8 days
Air Requirement	1450 ft^3/lb BOD
Clarifier Overflow Rate	526 gpd/ft^2
Clarifier Detention Time	2-3.6 hours
Denitrification (Fixed Film)	
Media Size	3 to 6 mm
Specific Surface Area	245 ft^2/ft^3
Hydraulic Loading Rate	5.9 gpm/ft^2
Methanol/NO_3-N Ratio	2:1 to 4:1
Bed Depth	15 ft
Detention Time (Empty Bed)	9.5 min
Operation	Downflow Packed Bed
Granular Carbon Adsorption	
Detention Time (Empty Bed)	35 min
Hydraulic Loading Rate	7 gpm/ft^2
Columns in Series	4
Carbon Size (Filtrasorb 300)	8 x 30 Mesh
Operation	Downflow Packed Bed
Filtration with Alum and Polymer	
Hydraulic Loading Rate	3 gpm/ft^2
Dual Media	
Coal (1.2-1.4 mm)	2.0 ft
Sand (0.6-0.7 mm)	1.0 ft
Alum	5 mg/l
Disinfection with Chlorine	
Detention Time	20 min
Residual	1 mg/l Free Available

Haloorganics--

Chloroform, bromodichloromethane, dibromochloromethane, bromoform, carbon tetrachloride, and 1,2-dichloroethane were determined in the influent and effluent. Levels were on the low side of the concentration range typically found in finished drinking water supplies.

Pesticides--

Twelve pesticides (from the DDT or parathion groups) were tested in the effluent and none exceeded EPA standards for drinking water.

EFFLUENT CONCENTRATION

The concentration process was based on the reverse osmosis-Donnan deionization technology as described in Section 3. The major problem in preparing the sample was reducing the inorganic content of the water. Due to the partial failure of the Donnan deionization system, alternate procedures--electrodialysis and closed loop dialysis--were considered for sample deionization. Electrodialysis was disadvantaged by an inconvenient temperature increase and associated control problem. Therefore, the sample was prepared using the closed loop dialysis method for overall inorganic reduction. The cation exchange system was used occasionally to balance cation levels, specifically, the exchange of Na^{+} for K^{+}. In addition, precipitative techniques were used to reduce levels of SO_4^{2-} and Ca^{2+} which were not easily handled by the membrane system.

Recovery of the Organic Soluble Fraction

The recovery calculation is based on TOC measurement. This estimation may have sizable error because (1) low-level TOC measurements (around 1 ppm) usually are not very accurate, and (2) effluent TOC was not measured throughout the concentration period, and values are based on those taken periodically during the pilot plant operation. The pilot plant data suggest a median value of about 2 mg/l, which was used for the recovery calculation.

The data in Table 14 reflect actual organic levels in the concentrate at various stages of the sample preparation sequence. As shown, mass retention was better in the final reverse osmosis concentration (94%) than in the initial reverse osmosis stage (53%), probably because most permeable organics have been removed in early steps.

In summary, an aqueous organic concentrate was prepared from the Blue Plains advanced waste treatment pilot plant effluent. Approximately 400,000 liters was concentrated to a final volume of 204 liters with a TOC level of approximately 704 mg/liter. Because the Donnan dialysis system did not function properly, several unanticipated process steps were necessary to prepare the sample. All the additional steps were shown to decrease the overall level of organics in the concentrate.

TABLE 14. ORGANIC LEVELS AT VARIOUS STAGES OF SAMPLE PREPARATION

Process Step	Sample Volume (liters)	Sample TOC (mg/l)	Mass of Organics (g)	Total Organic Mass Retention (%)	Step-Wise Organic Mass Retention (%)
Initial reverse osmosis concentration	757	530	402.1	53.0	53.0
Alkaline precipitation/ filtration	757	384	290.7	38.3	72.5
Closed-loop dialysis	757	260	196.8	26.0	67.7
Final reverse osmosis concentration	204	907	185.5	24.5	94.3
SO_4^{2-} precipitation/ filtration	204	704	144	19.0	77.6

TOXICOLOGICAL STUDIES

In Vivo Studies

The toxicological study of the concentrated recycled water was performed using CF1 mice. The main study was restricted to 90 days since the amount of the concentrated water was limited. This schedule was based on preliminary calculation of the daily water intake of the animal, minimum required number of animals needed for statistical analysis, and the need to study chronic exposure. Since the amount of the sample was limited, as many experiments as possible were carried out on the same group of animals without jeopardizing the individual experiments. All the *in vivo* toxicological experiments performed were listed in Table 9. Because most of the planned experiments had no precedent with regard to concentrated recycled water, it was important to perform these experiments in duplicate.

The plan of the studies was given in Tables 8 and 10. Not every animal in each study was used for all the experiments. The number of the animals used in each experiment is shown in Table 15.

Palatability Test--

Before starting the experiments a palatability test was run. The food was prepared as described in Section 3. The TOC level of the water was 292 mg/liter. Ten mice (B6C3F1), five males and five females, were fed the test concentrate. A second group of each sex received the same diet food containing deionized water; a third group received Wayne Meal. All food and water were provided fresh daily.

Food consumption was measured daily by weighing the food remaining in the bowl and then discarding it. Each animal was provided with an excess of feed to assure no starvation occurred. All mice were observed for seven days for signs of clinical effects. No evidence of toxicity from the test food was observed. No resistance to consume the test food was noted. The amount of food consumed by the mice on the three diets was approximately equal (Table 16).

TABLE 16. AVERAGE FOOD CONSUMPTION (g/mouse/day)

	Male	Female
Wayne Meal	11.3	9.8
Control Gel	10.8	9.1
Test Gel	10.6	8.9

All animals were weighed at the time of the first feeding, and again on days 3, 5 and 7. Results are given only for days 0 and 7. Weight gains were about the same among the three groups (Table 17).

TABLE 15. NUMBER OF MICE USED IN IN VIVO STUDIES

Test	Study P Male	P Female	I Male	I Female	II Male	II Female	III Male	III Female	IV Male	IV Female
General Physiology	50	100	200	200	100	100	50	50	50	-
Food Consumption	50	100	200	200	100	100	50	50	50	-
Body Weight	50	100	200	200	100	100	50	50	50	-
Hematology	-	-	84	86	50	50	-	-	-	-
Blood Chemistry	-	-	195	187	78	83	48	45	34	-
MFO Activity	-	-	95	96	48	50	-	-	-	-
Motor Activity	-	-	76	76	50	50	-	-	-	-
Reproduction	50	100	-	-	-	-	50	50	-	-
Dominant Lethal Mutation	50	-	50	50	-	-	-	-	-	-
Pathology	-	-	200	200	100	100	50	50	-	-

TABLE 17. AVERAGE BODY WEIGHT (g)

	Wayne Meal		Control Gel		Test Gel	
	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
Males	12.8	19.8	13.0	17.8	12.6	18.6
Females	11.6	17.8	12.0	17.8	11.6	16.6

Study P--

This preliminary experiment was carried out for 14 days. The purpose of this study was to test for general health effects or lethality at several TOC concentrations. The experiment was composed of 100 male mice and 200 female mice (aged 10 weeks) divided into 5 groups: A-control, B-water sample diluted 1:8 with deionized water, C-diluted 1:4, D-diluted 1:2, and E-undiluted concentrated water. One male and two females were put in each cage. Food consumption and general condition were observed daily and body weight on days 0, 3, 7, 10 and 14 recorded. Body weight and food consumption are given in Tables 18 and 19.

TABLE 18. MICE BODY WEIGHTS (g)

Day	0		7		14	
Group	Male	Female	Male	Female	Male	Female
A	35.3±1.9	22.9±2.1	33.2±2.7	25.2±2.2	34.0±2.1	29.0±3.5
B	35.0±2.1	22.1±1.8	33.0±1.4	24.9±1.8	33.4±1.6	30.7±3.2
C	33.4±3.0	22.0±1.6	31.6±2.5	25.8±1.1	32.0±2.4	31.9±2.9
D	30.5±2.5	20.8±2.9	31.4±1.6	25.1±2.1	34.7±4.4	29.3±3.1
E	33.2±2.7	21.1±1.4	33.0±3.2	23.1±1.8	34.7±4.4	29.3±3.1

TABLE 19. MEAN DAILY FOOD CONSUMPTION (g)

Group	Male	Number	Female	Number
A	7.8±0.7	10	7.9±0.6	20
B	7.2±0.9	10	7.7±0.7	20
C	7.2±0.5	10	7.7±0.2	20
D	7.5±1.2	10	7.8±0.3	20
E	7.9±0.7	10	7.9±0.5	20

Group B was exposed to about 10 mg of TOC per kilogram of body weight per day and Group E to about 100 mg TOC/kg/day. Humans drinking water with a TOC concentration of 1.0 mg/1 are exposed to 0.02-0.04 mg per kilogram of body weight per day. Thus, the mice were exposed to 500-5000 times the level humans are exposed to when drinking medium TOC water, and 50-500 times the exposure level for high-TOC drinking water (10 mg/1). No significant changes were observed in body weights or food intake for the mice exposed to high TOC water for two weeks.

Studies I through IV--

Study I was started with 500 animals [part of these are classified as Study IV (Table 8)]. As in Study P, these animals were quarantined for two weeks after arrival. Animals of Study II were the offspring of the females and males from Study P and were thus exposed to the concentrated water throughout gestation and lactation.

Studies I and II were carried out for 90 days; Study I from the age of five weeks and Study II from three weeks (weaning). Part of Study II was extended to 150 days and was designated Study III. The results of the tests performed during these studies are discussed in the following sections.

General Physiology--

Food consumption and clinical observations were recorded daily. Body weight was measured twice a week. No difference in body weights was apparent between the control animals and the experimental groups. A characteristic picture of growth can be seen in Tables 20 and 21. The daily food consumption (per kg body weight) decreased with age and was higher in females than in males because of changes in body weight. This difference in exposure per unit of body weight did not affect the toxicity, and no selective toxicity could be shown in the young or in the females. Lethality among the animals was low and could not be related to exposure of the experimental groups in any of the studies.

Food consumption was in the range of 200-600 g/day/kg body weight, which is equivalent to 100-300 g of water in the gel diet per day per kg body weight. Based on 700 mg/1 TOC in the undiluted water, the mice in the different groups were exposed to 12-140 mg TOC/day/kg body weight. Humans might typically be exposed to water containing up to 10 mg/1 of TOC. Based on daily water intake of 2 liters, human intake of TOC could be in the range of 0.02-0.4 mg TOC/kg/day. Thus, the mice in these studies were exposed to TOC doses 100-1000 times the expected human exposure.

Hematology--

Tables 22 and 23 list the data for hemoglobin, and red and white blood cell counts in Studies I and II. No differences were noted between the experimental groups and the controls.

Blood Chemistry and Tissue Weight--

Blood samples were taken by heart puncture from the mice on sacrifice day. About 50 mice, including an equal number of females and males from all groups, were sacrificed each day. The entire procedure took 2 weeks

TABLE 20. BODY WEIGHTS AND FOOD CONSUMPTION
(Study I - Females)

Group	N	Day	Body Weight (grams) $\bar{X}$	S.D	Food Consumption $\bar{X}$(g)	S.D	g/day/kg body weight
A	40	3	20.0	2.1	9.0	1.2	450.0
A	40	17	22.3	2.2	9.2	1.7	412.6
A	40	31	22.8	3.0	9.4	0.8	412.3
A	40	45	25.6	2.6	8.2	1.7	320.3
A	40	65	28.7	3.2	9.0	1.4	313.6
A	40	85	27.9	3.8	8.9	1.0	319.0
B	40	3	20.3	2.3	8.2	1.9	403.9
B	40	17	23.0	2.0	8.2	1.7	356.5
B	40	31	23.7	1.8	9.2	1.1	388.2
B	40	45	25.9	2.3			
B	40	65	28.9	3.2	9.3	1.4	321.8
B	40	85	27.5	3.6	8.9	1.3	323.6
C	40	3	21.3	1.8	7.3	2.4	342.7
C	40	17	23.2	1.5	8.9	1.7	383.6
C	40	31	23.6	1.5	10.0	0.0	423.7
C	39	45	25.3	1.8	8.7	1.8	343.9
C	39	65	27.6	3.7	8.9	1.5	322.5
C	39	85	27.0	3.6	9.0	1.8	333.3
D	40	3	20.1	1.9	9.3	1.5	462.7
D	40	17	21.0	2.3	9.8	0.6	466.7
D	40	31	23.2	2.7	9.4	1.1	405.2
D	39	45	24.1	1.8	8.2	1.2	340.2
D	40	65	26.3	4.0	8.4	1.3	319.4
D	40	85	26.6	4.6	8.4	2.1	315.8
E	39	3	20.0	1.8	9.4	1.3	470.0
E	39	17	21.7	1.6	9.9	0.6	456.2
E	39	31	23.6	1.7	9.1	1.3	385.6
E	39	45	25.1	1.9	9.0	1.2	358.6
E	39	65	25.9	2.2	9.3	0.9	359.1
E	39	85	27.3	3.3	8.9	1.8	326.0

TABLE 21. BODY WEIGHTS AND FOOD CONSUMPTION
(Study I - Males)

Group	N	Day	Body Weight (grams) $\bar{X}$	S.D	Mean Daily Food Consumption $\bar{X}$(g)	S.D	g/day/kg body weight
A	40	3	26.1	2.0	9.2	2.7	352.5
A	40	17	30.0	2.3	8.9	2.0	296.7
A	40	31	32.6	3.2	9.6	0.8	294.5
A	40	45	35.8	4.2	8.3	1.5	231.8
A	40	65	41.4	5.8	9.3	1.1	224.6
A	40	85	42.9	6.9	8.7	1.2	202.8
B	40	3	27.5	2.2	7.6	2.0	276.4
B	40	17	30.5	2.3	9.3	1.3	304.9
B	40	31	33.6	3.0	9.1	1.1	270.8
B	40	45	36.7	3.8	9.0	1.6	245.2
B	40	65	42.3	4.9	8.7	2.4	205.7
B	40	85	44.0	5.7	8.5	1.4	193.2
C	40	3	26.1	1.9	8.0	1.7	306.5
C	40	17	29.0	2.0	9.4	1.3	324.1
C	40	31	32.1	2.7	9.7	0.8	302.2
C	40	45	35.0	3.4	8.6	1.5	245.7
C	40	65	39.8	4.9	9.0	1.5	226.1
C	40	85	42.5	4.9	9.4	1.6	221.2
D	40	3	24.7	3.0	8.9	1.6	360.3
D	40	17	28.9	2.8	9.8	0.6	339.1
D	40	31	32.4	3.3	9.4	1.1	290.1
D	40	45	34.0	4.0	8.6	1.0	252.9
D	39	65	38.5	4.9	8.3	1.3	215.6
D	39	85	43.4	6.3	9.1	1.4	209.7
E	40	3	26.9	2.7	10.0	0.2	371.7
E	40	17	30.4	1.9	9.7	0.9	319.1
E	40	31	33.7	3.9	9.8	0.6	290.8
E	40	45	35.6	4.1	9.4	1.3	264.0
E	39	65	40.0	4.7	9.6	1.1	240.0
E	39	85	42.8	4.6	9.2	1.6	215.0

TABLE 22. HEMATOLOGICAL INDICES FOR MICE EXPOSED TO CONCENTRATED RECYCLED WATER (Study I)

Group	Sex	N	Hemoglobin (g/100 ml)		Red Blood Cells (10^6 cells/mm^3)		White Blood Cells (10^3 cells/mm^3)	
			M	SD	M	SD	M	SD
A	Male	17	15.57	0.97	8.79	0.52	10.75	3.01
A	Female	19	17.62	1.68	9.67	0.84	11.66	3.5
B	Male	16	15.74	0.97	9.15	0.54	12.13	4.36
B	Female	16	17.24	1.77	9.59	1.0	10.36	3.02
C	Male	16	16.11	1.32	9.15	0.75	13.3	5.63
C	Female	17	16.92	1.63	9.39	1.0	11.33	3.055
D	Male	18	16.07	0.99	9.41	0.61	11.93	2.29
D	Female	19	17.63	1.49	9.96	0.85	10.45	2.32
E	Male	17	16.03	1.36	9.81	0.69	12.8	2.86
E	Female	15	16.97	2.82	9.23	1.29	9.93	4.38

TABLE 23. HEMATOLOGICAL INDICES FOR MICE EXPOSED TO CONCENTRATED RECYCLED WATER (Study II)

Group	Sex	N	Hemoglobin (g/100 ml)		Red Blood Cells (10^6 cells/mm^3)		White Blood Cells (10^3 cells/mm^3)	
			M	SD	M	SD	M	SD
A	Male	10	16.23	1.09	9.3	0.56	12.23	2.42
A	Female	10	17.96	1.64	9.98	0.80	10.67	3.63
B	Male	10	15.83	0.84	9.1	0.52	12.74	3.8
B	Female	10	17.05	1.76	9.37	0.92	15.32	3.79
C	Male	10	15.55	1.59	8.95	0.86	14.79	2.02
C	Female	10	16.8	1.38	9.47	0.72	10.15	2.9
D	Male	10	15.97	0.91	8.93	0.53	12.95	2.48
D	Female	10	16.84	1.57	8.98	0.97	12.01	2.27
E	Male	10	16.1	0.75	9.18	0.56	13.13	3.10
E	Female	10	17.07	1.02	9.69	0.69	11.0	4.77

for Study I and about a week for the other studies. Serum samples from the mice were processed on an SMAC 20 Autoanalyzer (Technicon). Several preliminary trials were run to verify the quality and dilution of the sample and calibration of the instrument.

Results of the blood chemistry tests and the tissue weights for the various experimental groups are presented in Tables 24 through 51. The abbreviations for the different tests are explained in Section 3 (page 22). Results for the experimental groups which were found to be significantly different from the controls are marked by an asterisk (*). Tissue weight relative to body weight is designated by the letter R. These values should be multiplied by 0.001 to obtain the actual values. Tables 24 through 37 give the means, standard deviations, and number of individuals in each measurement. Tables 38 through 51 give the range of the results and the exact degree of significance (P value) for each test. Because of the large number of tests it was arbitrarily decided to regard as significant only those results which showed a P value ≤ 0.05 in more than one experimental group.

A general examination of the tables reveals that there are only a few results for the experimental groups that differ significantly from those for the control group ($P \leq 0.05$). In Study I (males), Na^+, Cl^-, K^+, and HCO_3^- were higher in a few experimental groups than in the control group. Such a combined change is logical, since the concentrations of these ions usually depend on each other. However, this effect was not repeated in the other studies. Males in Study II had lower urea values in the experimental groups and higher values of serum proteins. Glucose, triglycerides, and alkaline phosphatase were lower in the experimental groups than in the control males in Study III. The females of this group had lower values of cholesterol and urea but higher values of chloride. Almost none of these limited significant results have been repeated in two different studies.

Results were similar for the tissue weight. Only a few significant differences were noted. In Study I the males' brains were heavier in Groups D and E than in the control group. The spleens in the females of the Study I were smaller in the experimental groups than in the control. The lungs were larger in the experimental groups of males from Study II. In males and females from Study III, the spleens and adrenals were heavier in the experimental groups than in the control. No significant pathological results could be related to these tissue weight differences (see page 78).

There is very little back-up data available on the clinical chemistry of laboratory animals for most of the parameters tested, primarily because of sample volume limitations. Using the technology available today, a volume of 0.5 ml serum is needed to perform all 20 tests. Approximately 1 ml of blood is needed to produce 0.5 ml of serum. We had more than 95 percent success in drawing this amount of blood from the mice. We were able to find only one set of comparable data (for an unspecified strain of mice), which had been tested using a similar instrument and included the parameters examined in our study (W.H. Baum, Scientific Associates, Inc., St. Louis, Missouri 63123, personal communication). These results are given in Table 52. Although no standard deviation is given, some

TABLE 24. BLOOD CHEMISTRY RESULTS FOR STUDY I MALES

		Group A			Group B			Group C			Group D			Group E		
	Units	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N
Glu	mg/dl	227.75	86.12	24	252.92	50.86	24	231.44	68.45	25	230.54	72.79	28	217.07	69.45	28
Chol	mg/dl	184.42	31.82	24	178.58	29.18	24	202.40*	29.59	25	194.86	22.99	28	199.50	23.06	28
Trig	mg/dl	35.53	25.06	36	27.41	12.63	41	28.67	19.11	42	28.08	20.60	38	37.57	19.77	37
TP	g/dl	4.57	0.44	24	4.53	0.32	24	4.73	0.28	25	4.64	0.24	28	4.56	0.15	28
Alb	g/dl	2.57	0.28	36	2.54	0.21	41	2.56	0.30	42	2.58	0.19	38	2.58	0.18	38
BUN	mg/dl	22.67	6.01	36	21.56	4.46	41	20.95	5.10	42	21.42	5.22	38	20.32	4.55	38
UA	mg/dl	3.34	1.84	25	3.89	2.14	24	2.95	2.26	25	2.81	0.98	28	2.43*	1.16	28
Bili**	mg/dl	0.03	0.07	36	0.27	1.40	41	0.03	0.08	42	0.03	0.07	38	0.03	0.07	38
Creat	mg/dl	0.44	0.16	35	0.41	0.11	40	0.39	0.13	40	0.42	0.20	38	0.61	1.12	37
Na	meq/l	138.17	11.51	24	141.83	4.33	24	141.68	3.30	25	145.07	4.54	28	144.86*	5.40	28
K	meq/l	6.21	1.29	35	5.95	0.93	40	5.68*	0.97	42	6.27*	4.33	38	5.91	0.92	38
Cl	meq/l	85.51	14.03	37	90.10	7.27	41	88.24	9.30	42	92.58	7.87	38	92.63*	6.33	38
CO_2	meq/l	19.11	3.66	36	22.54*	2.93	41	20.33	2.61	42	22.32*	2.13	38	21.05*	2.74	38
Ca	mg/dl	8.52	0.66	36	8.79*	0.46	41	8.63	0.65	42	8.73	0.37	38	8.62	0.32	38
P	mg/dl	7.38	1.41	23	6.93	0.96	24	7.40	1.03	25	7.11	1.47	28	7.53	1.19	28
AP	U/l	70.06	11.29	36	67.51	9.40	41	82.45	90.11	42	74.42	18.56	38	73.26	10.86	38
LDH	U/l	345.39	141.41	31	303.00	156.06	40	324.71	175.51	41	331.15	153.28	33	327.15	151.86	31
SGOT	U/l	88.67	45.93	33	75.92	42.63	40	82.40	41.42	40	80.79	39.71	38	85.39	49.01	36
SGPT	U/l	39.67	25.84	36	33.52	25.54	38	42.19	32.26	42	31.19	19.44	37	42.70	39.25	37
CPK	U/l	365.06	349.42	36	415.70	491.43	40	326.91	307.52	42	323.89	266.34	37	410.16	459.91	38

*Significant ($p \leq 0.05$)

**In Group B one bilirubin was 9 mg.

TABLE 25. TISSUE WEIGHTS FOR STUDY I MALES

		Group A			Group B			Group C			Group D			Group E		
	Units	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N
Heart	mg	193.2	25.5	40	187.7	36.4	40	188.6	24.2	40	193.6	21.9	41	192.1	23.8	39
Lungs	mg	207.2	26.7	40	220.9	47.0	40	212.2	32.8	40	220.6	51.4	41	217.0	42.6	39
Spleen	mg	100.2	115.8	40	89.2	57.7	40	82.7	22.7	40	81.2	41.8	41	76.05	17.2	39
Liver	mg	1508.8	266.0	26	1597.4	224.0	23	1525.6	247.3	25	1619.4	283.6	31	1595.2	229.8	29
Kidney	mg	554.0	69.0	40	574.3	81.8	40	541.3	70.8	40	568.7	62.4	40	538.1	113.1	39
Adrenals	mg	5.7	2.0	39	5.6	1.7	40	7.0	14.0	40	5.0	1.7	41	5.6	2.0	38
Brain	mg	404.8	40.41	26	425.0	38.6	24	418.4	37.3	25	425.3*	27.0	30	433.6*	26.9	29
Testes	mg	236.5	24.0	40	236.4	35.9	40	235.0	26.7	40	229.5	24.4	41	231.9	22.5	39
Body Weight	g	43.15	6.38	40	44.33	5.72	40	43.48	5.22	40	43.37	6.10	41	44.0	5.1	39
R. Heart	10^{-3}	4.54	0.65	40	4.26	0.85	40	4.38	0.58	40	4.53	0.69	41	4.41	0.66	39
R. Lungs	10^{-3}	4.89	0.78	40	5.03	1.12	40	4.94	0.95	40	5.15	1.39	41	4.97	0.93	39
R. Spleen	10^{-3}	2.36	2.90	40	2.02	1.28	40	1.93	0.61	40	1.91	1.15	41	1.75	0.41	39
R. Liver	10^{-3}	34.80	5.95	26	35.91	4.61	23	35.33	4.76	25	36.81	4.95	31	36.00	11.63	29
R. Kidney	10^{-3}	13.05	1.84	40	13.11	2.22	40	12.52	1.48	40	13.36	2.0	40	13.41	2.99	39
R. Ardrenals	10^{-3}	0.13	0.05	39	0.13	0.04	40	0.17	0.38	40	0.12	0.04	41	0.13	0.04	38
R. Brain	10^{-3}	9.48	1.83	26	9.72	1.58	24	9.77	1.30	25	9.89	1.66	30	9.86	1.16	29
R. Testes	10^{-3}	5.60	0.96	40	5.39	0.95	40	5.46	0.75	40	5.38	0.90	41	5.33	0.70	39

TABLE 26. BLOOD CHEMISTRY RESULTS FOR STUDY I FEMALES

		Group A			Group B			Group C			Group D			Group E		
	Units	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N
Glu	mg/dl	148.23	67.16	26	187.21*	69.92	28	142.86	72.84	28	124.83	58.05	17	161.85	67.84	26
Chol	mg/dl	138.52	21.18	37	133.95	16.51	39	142.58	22.80	38	137.78	34.49	37	135.50	23.61	36
Trig	mg/dl	15.46	10.49	26	15.71	9.31	28	18.79	12.50	28	11.29	6.71	17	13.54	8.28	26
TP	g/dl	4.52	0.44	37	4.63	0.22	39	4.64	0.40	38	4.55	0.38	37	4.55	0.56	36
Alb	g/dl	2.59	0.29	37	2.62	0.47	39	2.70	0.27	38	2.63	0.31	37	2.64	0.39	36
BUN	mg/dl	21.69	7.75	26	25.71	9.09	28	23.21	10.40	28	31.18*	12.47	17	23.11	8.98	27
UA	mg/dl	3.23	2.35	30	2.79	1.70	28	2.96	2.13	28	2.88	1.99	18	2.43	1.74	27
Bili	mg/dl	0.04	0.08	37	0.08	0.29	39	0.04	0.08	38	0.06	0.10	37	0.06	0.09	36
Creat	mg/dl	0.32	0.14	26	0.36	0.10	28	0.36	0.11	28	0.28	0.10	17	0.33	0.11	27
Na	meq/l	144.62	7.11	26	142.68	5.53	28	145.93	8.17	28	147.65	9.03	17	146.22	10.95	27
K	meq/l	5.78	1.22	37	5.92	0.96	39	5.71	0.88	38	6.10	1.10	36	5.56	1.08	36
Cl	meq/l	85.51	24.24	37	92.10	11.59	39	93.62	12.18	38	96.27*	11.80	37	92.39	17.97	36
CO_2	meq/l	17.95	4.07	37	18.36	3.55	39	17.97	4.00	38	18.00	4.03	37	17.94	3.93	36
Ca	mg/dl	8.73	0.83	37	8.79	0.36	39	8.73	1.00	38	8.84	0.63	37	8.07	2.27	38
P	mg/dl	7.87	1.52	37	7.31	1.55	39	7.71	0.98	38	7.77	1.39	37	1.56	1.36	36
AP	U/l	123.97	26.33	37	118.56	25.04	39	122.05	29.44	38	127.30	26.60	37	127.78	26.21	36
LDH	U/l	368.56	225.69	36	358.62	213.82	39	309.45	166.51	38	353.74	189.76	34	311.25	224.85	36
SGOT	U/l	138.50	144.01	36	145.68	131.83	38	112.74	64.81	38	161.66	135.87	35	127.50	105.35	36
SGPT	U/l	34.00	37.36	34	33.23	18.01	39	26.27	17.21	37	35.89	29.31	37	28.72	29.17	36
CPK	U/l	490.59	559.70	37	482.89	485.93	38	392.63	409.74	38	372.97	280.02	35	306.22	306.28	36

TABLE 27. TISSUE WEIGHTS FOR STUDY I FEMALES

		Group A			Group B			Group C			Group D			Group E		
	Units	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N
Heart	mg	162.29	26.22	40	152.70	20.12	40	150.34*	23.97	39	150.72	23.66	29	156.32	23.89	38
Lungs	mg	189.46	30.04	40	190.83	29.05	40	187.75	27.61	39	178.87	36.56	29	188.02	31.48	38
Spleen	mg	85.23	18.99	40	84.45	18.17	40	80.92	27.32	38	74.60*	17.62	29	74.36*	16.34*	38
Liver	mg	1169.00	178.91	30	1200.67	219.18	30	1149.38	173.85	28	996.84*	154.74	19	1156.71	163.91	28
Kidney	mg	348.75	70.75	40	343.75	49.39	40	344.26	64.71	39	334.12	50.39	29	328.89	59.07	38
Adrenals	mg	12.60	15.27	40	9.89	3.24	40	8.84	2.29	39	8.83	2.62	29	9.82	2.46	38
Brain	mg	453.25	71.91	40	443.50	42.88	40	447.69	43.56	39	437.43	38.80	29	441.16	39.61	38
Ovaries	mg	27.95	9.68	40	27.07	10.08	39	28.88	16.06	39	24.28	10.24	28	25.75	9.6	38
Body Weight	g	28.58	4.01	40	28.08	3.68	40	27.33	4.15	39	26.52*	3.48	29	27.45	3.47	38
R. Heart	10^{-3}	5.73	0.94	40	5.49	0.81	40	5.54	0.65	39	5.74	0.95	29	5.74	0.83	38
R. Lungs	10^{-3}	6.71	1.21	40	6.85	1.05	40	6.97	1.05	39	6.80	1.30	29	6.91	1.16	38
R. Spleen	10^{-3}	3.00	0.62	40	3.02	0.59	40	2.93	0.80	38	2.81	0.56	29	2.71*	0.46	38
R. Liver	10^{-3}	40.63	4.26	30	42.58	6.67	30	41.51	5.70	28	38.66	3.75	19	41.58	6.57	28
R. Kidney	10^{-3}	12.31	2.49	40	12.34	1.77	40	12.64	1.64	39	12.70	1.75	29	12.15	2.48	38
R. Adrenals	10^{-3}	0.44	0.50	40	0.36	0.13	40	0.33	0.09	39	0.34	0.10	29	0.36	0.10	38
R. Brain	10^{-3}	16.06	2.82	40	15.96	1.93	40	16.76	2.96	39	16.68	2.01	29	16.31	2.37	38
R. Ovaries	10^{-3}	0.92	0.34	40	0.97	0.36	39	1.05	0.54	39	0.90	0.31	28	0.94	0.33	38

TABLE 28. BLOOD CHEMISTRY RESULTS FOR STUDY II MALES

		Group A			Group B			Group C			Group D			Group E		
	Units	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N
Glu	mg/dl	217.22	86.41	18	246.24	83.97	17	247.87	85.69	15	233.2	85.57	15	243.40	107.80	11
Chol	mg/dl	175.56	25.12	18	224.24	140.45	17	191.29	20.30	14	193.20	29.30	15	183.20	22.73	10
Trig	mg/dl	49.59	23.59	17	47.83	24.99	18	42.71	15.86	14	58.00	17.17	15	37.45	18.93	11
TP	g/dl	4.72	0.37	18	4.95	0.37	17	4.96*	0.21	14	4.95*	0.24	14	4.92	0.25	10
Alb	g/dl	2.91	0.26	18	3.02	0.37	18	3.09	0.33	14	2.96	0.24	15	2.98	0.21	11
BUN	mg/dl	23.89	5.72	18	23.56	5.84	18	16.53*	2.88	15	18.80*	4.40	15	21.09	4.76	11
UA	mg/dl	2.64	1.17	18	2.49	1.17	18	2.01	0.99	16	3.36	1.54	15	2.92	1.27	10
Bili	mg/dl	0.02	0.07	18	0.00	0.00	18	0.03	0.07	14	0.01	0.05	15	0.00	0.00	11
Creat	mg/dl	0.46	0.17	18	0.46	0.09	18	0.46	0.09	14	0.53	0.15	15	0.51	0.10	11
Na	meq/l	153.11	7.04	18	155.06	2.75	18	156.14	3.80	14	153.67	4.03	15	155.2	2.70	11
K	meq/l	6.11	0.90	18	6.04	0.92	16	5.83	0.93	14	6.77	1.05	14	6.95*	0.76	11
Cl	meq/l	99.57	6.64	18	102.11	3.97	18	102.80	4.33	15	95.63	15.34	16	102.18	4.24	11
CO_2	meq/l	20.00	2.47	18	19.44	2.15	18	21.07	3.01	15	18.38	3.88	16	18.36	3.98	11
Ca	mg/dl	8.71	0.62	18	9.02	0.57	18	8.76	0.33	15	9.08	0.35	15	8.76	0.29	11
P	mg/dl	7.91	1.17	18	8.39	1.17	17	7.43	0.85	15	9.16*	2.15	15	8.30	1.17	10
AP	U/l	91.44	15.06	18	89.33	13.00	18	83.57	8.78	14	84.13	11.92	15	89.45	13.57	11
LDH	U/l	521.73	173.50	15	618.83	205.70	18	580.29	236.68	14	539.38	152.87	13	560.18	222.46	11
SGOT	U/l	111.78	43.68	18	156.33	89.19	18	158.86*	63.69	14	128.80	34.02	15	156.91	88.70	11
SGPT	U/l	52.89	42.00	18	90.33	97.61	18	67.14	37.46	14	71.47	38.55	15	87.09	76.19	11
CPK	U/l	478.11	361.75	18	432.56	229.08	18	454.71	197.27	14	330.67	159.04	15	380.91	241.05	11

TABLE 29. TISSUE WEIGHTS FOR STUDY II MALES

	Units	Group A			Group B			Group C			Group D			Group E		
		M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N
Heart	mg	186.37	12.95	19	191.41	23.49	21	175.54*	17 18	19	189.32	22.53	19	179.81	25.12	15
Lungs	mg	185.86	27.34	19	218.51*	37.66	21	209.82*	22.93	19	207.12*	21.81	19	193.81	25.41	15
Spleen	mg	61.62	13.17	19	78.13*	30.39	21	65.13	20.97	19	76.68*	12.04	19	65.99	18.95	15
Liver	mg	1399.04	249.06	19	1548.86	333.04	20	1379.97	310.54	19	1322.38	215.66	19	1387.38	200.85	15
Kidney	mg	495.49	128.33	19	544.75	107.31	21	477.55	62.59	19	486.82	135.20	19	483.21	82.71	15
Adrenals	mg	5.36	2.87	19	3.99	2.56	21	3.71*	1.81	19	6.80	2.09	19	4.68	1.70	15
Brain	mg	436.02	61.77	19	447.99	38.64	20	420.09	47.22	19	420.51	56.96	19	421.61	52.44	15
Testes	mg	201.01	47.66	19	212.20	29.92	21	215.12	21.81	19	232.51*	23.47	18	201.15	22.57	15
Body Weight	g	34.63	5.13	19	38.22	5.79	18	38.79*	5.59	19	38.21	6.47	19	37.33	5.68	15
R. Heart	10^{-3}	5.49	0.84	19	4.97*	0.54	18	4.57*	0.50	19	5.04	0.79	19	4.89	0.89	15
R. Lungs	10^{-3}	5.38	0.43	19	5.57	0.85	18	5.48	0.74	19	5.54	0.96	19	5.26	0.74	15
R. Spleen	10^{-3}	1.81	0.47	19	1.88	0.37	18	1.70	0.56	19	2.04	0.97	18	1.75	0.31	15
R. Liver	10^{-3}	40.45	4.23	19	40.09	5.48	18	35.61*	5.24	19	34.82*	3.54	19	37.40*	3.70	15
R. Kidney	10^{-3}	14.39	3.62	19	14.13	2.82	18	12.45*	1.71	19	12.86	3.34	19	13.12	2.39	15
R. Ardenals	10^{-3}	0.17	0.11	19	0.11	0.09	18	0.10*	0.05	19	0.18	0.06	19	0.13	0.05	15
R. Brain	10^{-3}	12.91	2.95	19	11.92	1.94	18	11.04*	1.89	19	11.18*	1.82	19	11.57	2.51	15
R. Testes	10^{-3}	5.83	1.51	19	5.60	0.97	18	5.62	0.76	19	6.19	1.19	18	5.49	0.88	15

TABLE 30. BLOOD CHEMISTRY RESULTS FOR STUDY II FEMALES

		Group A			Group B			Group C			Group D			Group E		
	Units	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N
Glu	mg/dl	197.76	102.23	17	159.75	73.48	16	154.18	81.58	11	172.22	62.18	18	221.68	100.53	19
Chol	mg/dl	137.24	25.86	17	127.76	25.42	17	139.82	18.43	11	130.95	12.44	19	138.00	14.94	18
Trig	mg/dl	42.13	13.38	15	34.06	20.50	16	37.73	25.78	11	37.00	14.48	18	32.82	16.99	19
TP	g/dl	5.11	0.21	17	4.8	0.67	17	5.04	0.28	11	4.98	0.33	19	5.13	0.43	19
Alb	g/dl	3.18	0.32	17	3.08	0.52	17	3.05	0.37	11	2.98	0.29	19	3.08	0.38	19
BUN	mg/dl	18.71	5.96	17	18.25	5.46	16	14.18*	2.75	11	19.33	7.36	18	19.79	6.89	19
UA	mg/dl	2.93	1.25	17	2.38	1.70	18	2.44	1.19	11	2.21	1.15	18	2.62	1.14	19
Bili	mg/dl	0.01	0.05	17	0.02	0.07	17	0.02	0.06	11	0.01	0.05	19	0.01	0.05	19
Creat	mg/dl	0.48	0.12	17	0.49	0.15	16	0.45	0.09	11	0.50	0.14	18	0.51	0.12	19
Na	meq/l	156.18	4.21	15	150.00	21.38	16	152.91	5.89	11	154.89	3.83	18	156.32	5.97	19
K	meq/l	6.26	0.86	16	6.14	1.13	17	6.83	1.40	11	5.75	0.86	19	6.35	1.15	19
Cl	meq/l	95.88	13.75	16	98.82	20.51	17	93.73	15.89	11	103.68*	4.96	19	94.84	15.34	19
CO_2	meq/l	17.06	4.48	17	16.71	6.20	17	18.36	3.08	11	16.84	4.44	19	16.84	3.08	19
Ca	mg/dl	9.05	0.47	17	8.68	1.11	17	9.13	0.68	11	9.22	0.45	19	9.25	0.63	19
P	mg/dl	8.60	1.63	11	8.20	2.19	17	7.60	1.36	11	8.15	1.48	19	9.19	2.22	19
AP	U/l	141.53	27.02	17	136.71	24.24	17	138.18	43.38	11	126.74	17.99	19	140.89	24.68	18
LDH	U/l	522.27	232.63	15	471.88	131.05	16	412.09	273.71	11	446.74	151.75	19	414.11	204.66	19
SGOT	U/l	196.35	137.08	17	210.47	92.68	17	224.00	201.13	11	180.63	114.28	19	163.58	99.91	19
SGPT	U/l	64.59	84.95	17	64.71	33.56	17	82.91	78.77	11	59.05	44.40	19	43.58	33.44	19
CPK	U/l	460.35	246.18	17	458.25	254.21	16	380.36	462.97	11	368.74	238.60	19	383.89	286.28	19

TABLE 31. TISSUE WEIGHTS FOR STUDY II FEMALES

	Units	Group A			Group B			Group C			Group D			Group E		
		M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N
Heart	mg	144.39	19.69	19	150.24	18.46	19	141.60	22.67	19	155.81	21.54	20	146.97	19.30	23
Lungs	mg	178.36	29.35	19	192.88	25.50	19	177.09	24.57	19	189.84	28.61	20	176.36	27.12	23
Spleen	mg	65.92	20.20	19	72.88	17.41	19	85.80	85.82	19	77.84	20.39	20	73.93	34.43	23
Liver	mg	1131.69	192.92	19	1102.42	170.43	19	1071.37	205.21	19	1143.67	185.61	20	1076.47	169.29	23
Kidney	mg	333.26	86.18	19	300.06	73.53	19	287.80	58.45	19	308.49	62.19	20	297.19	42.23	23
Adrenals	mg	9.32	2.92	19	9.30	2.70	19	8.45	1.74	19	10.73	1.63	20	9.91	3.44	23
Brain	mg	435.14	39.59	19	449.64	78.66	19	427.74	52.88	19	439.35	27.91	20	424.99	41.02	23
Ovaries	mg	21.04	9.78	19	23.99	9.17	19	21.47	8.18	19	26.08	6.83	20	21.63	9.32	23
Body Weight	g	26.84	4.89	19	25.53	3.88	19	25.05	4.12	19	25.75	4.19	20	24.52	3.88	23
R. Heart	10^{-3}	5.47	0.80	19	5.96	0.84	19	5.70	0.65	19	6.14*	0.94	20	6.05*	0.75	23
R. Lungs	10^{-3}	6.73	0.95	19	7.62*	0.94	19	7.19	1.09	19	7.47*	1.22	20	7.26	0.99	23
R. Spleen	10^{-3}	2.44	0.55	19	2.82*	0.58	19	3.29	2.67	19	3.01*	0.63	20	3.00	1.29	23
R. Liver	10^{-3}	42.49	5.22	19	43.33	4.69	19	43.08	6.86	19	44.73	5.99	20	44.19	4.74	23
R. Kidney	10^{-3}	12.65	4.05	19	11.78	2.73	19	11.75	2.82	19	12.22	2.85	20	12.24	1.64	23
R. Adrenals	10^{-3}	0.35	0.11	19	0.37	0.11	19	0.34	0.08	19	0.42*	0.08	20	0.40	0.12	23
R. Brain	10^{-3}	16.61	2.60	19	17.80	3.45	19	17.64	4.36	19	17.44	2.73	20	17.72	3.22	23
R. Ovaries	10^{-3}	0.79	0.63	19	0.95	0.37	19	0.85	0.30	19	1.02*	0.27	20	0.88	0.34	23

TABLE 32. BLOOD CHEMISTRY RESULTS FOR STUDY III MALES

	Units	Group A			Group B			Group C			Group D			Group E		
		M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N
Glu	mg/dl	310.00	60.90	10	193.75*	65.42	8	208.40*	55.89	10	240.40	91.92	10	177.56*	102.73	9
Chol	mg/dl	183.00	16.25	10	185.75	28.63	8	195.80	18.15	10	183.60	28.19	10	172.44	29.70	9
Trig	mg/dl	58.40	25.23	10	35.00	20.70	8	56.20	24.33	10	35.60*	10.70	10	32.89*	16.92	9
TP	g/dl	4.50	0.19	10	4.58	0.17	8	4.64	0.18	10	4.36	0.21	10	4.44	0.13	9
Alb	g/dl	2.58	0.06	10	2.63	0.13	8	2.64	0.13	10	2.48	0.14	10	2.51	0.11	9
BUN	mg/dl	21.40	3.66	10	21.00	2.62	8	17.40*	2.68	10	19.40	4.53	10	18.67	3.16	9
UA	mg/dl	3.04	1.34	10	2.95	0.86	8	2.76	0.93	10	2.12	0.64	10	2.38	0.61	9
Bili	mg/dl	0.00	0.00	10	0.05	0.09	8	0.00	0.00	10	0.02	0.06	10	0.02	0.07	9
Creat	mg/dl	0.40	0.09	10	0.30	0.15	8	0.44	0.21	10	0.38	0.06	10	0.49	0.18	9
Na	meq/l	142.80	2.53	10	141.75	2.92	8	140.60*	1.90	10	141.80	2.90	10	141.56	1.94	9
K	meq/l	6.47	1.51	10	5.78	0.47	8	5.60	0.28	10	5.84	0.52	10	5.69	0.47	9
Cl	meq/l	89.60	3.98	10	88.25	5.18	8	85.80*	2.39	10	90.40	3.24	10	90.22	3.67	9
CO_2	meq/l	20.80	2.53	10	23.00	2.83	8	22.40	2.07	10	21.80	4.05	10	20.89	2.85	9
Ca	mg/l	8.12	0.72	10	7.78	0.31	8	7.88	0.30	10	7.78	0.26	10	7.89	0.23	9
P	mg/dl	7.64	1.87	10	6.65	1.13	8	6.46	1.24	10	7.44	1.17	10	7.71	0.89	9
AP	U/l	74.60	6.19	10	68.25	10.71	8	73.00	14.09	10	64.40*	8.93	10	64.89*	9.07	9
LDH	U/l	378.00	205.59	10	462.50	178.84	8	437.00	183.27	10	427.60	183.19	10	423.11	183.46	9
SGOT	U/l	93.80	36.52	10	93.00	20.65	8	106.20	40.29	10	89.80	29.88	10	76.44	37.36	9
SGPT	U/l	52.80	32.93	10	44.00	20.11	8	74.00	47.10	10	66.20	61.79	10	53.33	40.89	9
CPK	U/l	261.40	104.07	10	215.50	155.32	8	341.40	197.20	10	337.40	262.37	10	170.89	83.63	9

TABLE 33. TISSUE WEIGHTS FOR STUDY III MALES

		Group A			Group B			Group C			Group D			Group E		
	Units	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N
Heart	mg	184.00	24.59	10	168.00	27.81	10	181.00	42.28	10	187.00	26.59	10	183.33	26.93	9
Lungs	mg	219.00	32.13	10	208.00	73.30	10	185.00	55.03	10	207.00	26.27	10	193.33	41.83	9
Spleen	mg	65.32	17.59	10	71.61	25.47	10	78.84	16.80	10	85.02*	16.48	10	87.36*	20.58	9
Liver	mg	1517.50	521.12	10	1450.00	414.35	10	1478.00	228.95	10	1440.00	239.21	10	1597.78	369.18	9
Kidney	mg	582.00	72.54	10	552.00	65.96	10	576.00	111.77	10	560.00	106.35	10	567.78	64.96	9
Adrenals	mg	6.38	2.11	10	6.14	1.83	10	11.22*	5.03	10	10.89*	5.39	10	8.60	4.22	9
Brain	mg	422.00	22.01	10	445.00	28.77	10	407.00	40.84	10	433.00	34.01	10	407.78	27.74	9
Testes	mg	210.00	9.43	10	215.00	41.70	10	219.00	45.57	10	202.00	26.58	10	182.22*	31.93	9
Body Weight	g	43.60	3.44	10	40.80	10.82	10	43.50	7.89	10	40.70	7.21	10	44.56	6.33	9
R. Heart	10^{-3}	4.24	0.63	10	4.23	0.57	10	4.14	0.45	10	4.69	0.73	10	4.13	0.45	9
R. Lungs	10^{-3}	5.04	0.76	10	5.08	1.18	10	4.25*	0.89	10	5.16	0.57	10	4.36	0.88	9
R. Spleen	10^{-3}	1.51	0.48	10	1.75	0.34	10	1.81	0.22	10	2.09*	0.16	10	1.95*	0.33	9
R. Liver	10^{-3}	34.34	11.17	10	35.68	5.09	10	34.37	4.30	10	35.77	4.40	10	35.56	3.76	9
R. Kidney	10^{-3}	13.33	1.05	10	14.04	2.27	10	13.30	1.53	10	14.00	2.62	10	12.93	2.03	9
R. Adrenals	10^{-3}	0.15	0.05	10	0.17	0.08	10	0.25*	0.09	10	0.28*	0.13	10	0.20	0.10	9
R. Brain	10^{-3}	9.75	1.10	10	11.51	2.55	10	9.61	1.81	10	10.91	1.75	10	9.33	1.49	9
R. Testes	10^{-3}	4.85	0.46	10	5.41	0.88	10	5.13	1.12	10	5.03	0.51	10	4.13*	0.80	9

TABLE 34. BLOOD CHEMISTRY RESULTS FOR STUDY III FEMALES

		Group A			Group B			Group C			Group D			Group E		
	Units	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N
Glu	mg/dl	216.89	51.39	9	181.40	42.84	10	167.40	54.19	10	145.11*	53.66	9	126.86*	43.63	7
Chol	mg/dl	152.44	20.51	9	134.20	22.10	10	121.60*	20.08	10	139.11	13.93	9	120.86*	15.09	7
Trig	mg/dl	23.11	11.80	9	21.00	8.18	10	33.90	29.28	10	20.00	1.28	9	14.86	3.02	7
TP	g/dl	4.53	0.41	9	4.48	0.19	10	4.48	0.22	10	4.42	0.25	9	4.49	0.16	7
Alb	g/dl	2.56	0.22	9	2.64	0.13	10	2.64	0.16	10	2.60	0.20	9	2.60	0.12	7
BUN	mg/dl	20.22	4.41	9	12.00*	2.49	10	14.40	7.23	10	13.11*	3.48	9	15.14*	2.80	7
UA	mg/dl	2.78	1.31	9	3.00	1.08	10	2.62	1.02	10	2.98	0.94	9	2.51	1.19	7
Bili	mg/dl	0.00	0.00	9	0.00	0.00	10	0.02	0.06	10	0.02	0.07	9	0.00	0.00	7
Creat	mg/dl	0.33	0.14	9	0.32	0.32	10	0.54*	0.21	10	0.47	0.17	9	0.54*	0.10	7
Na	meq/l	142.67	2.45	9	141.80	2.39	10	140.20*	2.57	10	143.33	3.16	9	142.57	2.76	7
K	meq/l	5.62	0.64	9	5.26	0.45	10	5.24	0.40	10	5.40	0.90	9	6.33	1.68	7
Cl	meq/l	87.11	3.62	9	90.00	6.11	10	88.20	3.05	10	91.56*	4.98	9	94.00*	4.00	7
CO_2	meq/l	30.40	21.08	9	22.00	2.67	10	19.80	3.46	10	20.00	3.87	9	16.86	4.14	7
Ca	mg/dl	8.40	0.46	9	8.12	0.22	10	8.20	0.33	10	7.89*	0.52	9	8.31	0.62	7
P	mg/dl	6.02	1.24	9	5.62	1.44	10	6.14	2.51	10	6.71	1.53	9	7.14	1.91	7
AP	U/l	107.33	19.67	9	127.60	23.07	10	124.60	72.79	10	128.44	28.89	9	94.29	13.64	7
LDH	U/l	287.56	129.02	9	268.60	137.01	10	250.22	133.63	10	417.33	205.02	9	350.43	137.52	7
SGOT	U/l	105.11	94.62	9	106.80	66.86	10	121.80	87.86	10	140.22	98.39	9	152.00	53.77	7
SGPT	U/l	51.56	71.56	9	35.80	31.81	10	55.00	48.92	10	48.89	36.85	9	55.14	30.96	7
CPK	U/l	141.56	64.40	9	167.00	125.11	10	277.00	231.08	10	256.00	172.96	9	244.86	145.95	7

TABLE 35. TISSUE WEIGHTS FOR STUDY III FEMALES

		Group A			Group B			Group C			Group D			Group E		
	Units	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N
Heart	mg	132.00	36.15	10	153.00	31.64	10	150.00	40.83	10	146.00	17.76	10	155.00	17.16	10
Lungs	mg	186.92	26.19	10	197.00	29.08	10	193.00	47.39	10	204.44	22.97	10	191.00	26.85	10
Spleen	mg	59.54	18.46	10	60.13	21.71	10	92.88*	28.62	10	77.69	25.86	10	80.58*	18.26	10
Liver	mg	1367.00	186.61	10	1354.00	173.79	10	1514.00	319.83	10	1298.20	77.21	10	1269.00	112.29	10
Kidney	mg	317.00	41.91	10	369.00*	54.66	10	344.00	84.35	10	303.00	38.89	10	323.00	69.77	10
Adrenals	mg	7.60	3.67	10	12.09*	4.66	10	15.89*	4.29	10	15.46*	9.06	10	11.61*	4.48	10
Brain	mg	423.10	37.69	10	432.00	21.50	10	426.00	38.06	10	391.00	35.10	10	395.00	66.71	10
Ovaries	mg	18.55	6.47	10	25.35	9.31	10	29.63*	7.83	10	21.76	9.37	10	24.85	7.13	10
Body Weight	g	30.90	5.30	10	34.80	3.88	10	35.90*	5.02	10	29.40	3.60	10	31.70	4.64	10
R. Heart	10^{-3}	4.28	1.11	10	4.44	1.04	10	4.26	1.26	10	5.02	0.75	10	4.96	0.74	10
R. Lungs	10^{-3}	6.22	1.31	10	5.71	0.94	10	5.44	1.31	10	6.92	0.42	10	6.07	0.70	10
R. Spleen	10^{-3}	1.96	0.68	10	1.75	0.66	10	2.58	0.75	10	2.67	0.91	10	2.54*	0.44	10
R. Liver	10^{-3}	44.74	5.30	10	39.00*	3.88	10	43.28	14.04	10	44.75	5.94	10	40.52	4.78	10
R. Kidney	10^{-3}	10.43	1.45	10	10.63	1.36	10	9.68	2.39	10	10.50	2.14	10	10.34	2.53	10
R. Adrenals	10^{-3}	0.24	0.11	10	0.35	0.15	10	0.45*	0.14	10	0.56*	0.39	10	0.38	0.18	10
R. Brain	10^{-3}	14.25	3.91	10	12.57	1.72	10	12.05	1.73	10	13.55	2.51	10	12.75	3.11	10
R. Ovaries	10^{-3}	0.60	0.18	10	0.75	0.34	10	0.82*	0.17	10	0.76	0.37	10	0.80	0.26	10

TABLE 36. BLOOD CHEMISTRY RESULTS FOR STUDY IV MALES

	Units	Group A			Group B			Group C			Group D			Group E		
		M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N
Glu	mg/dl	153.78	215.26	9	80.50	14.96	8	-	-	-	84.00	23.83	8	90.00	38.42	9
Chol	mg/dl	146.89	18.00	9	150.00	20.67	8	-	-	-	143.23	12.69	8	145.11	7.42	9
Trig	mg/dl	30.44	9.53	9	37.50	10.07	8	-	-	-	34.50	7.31	8	32.00	14.67	9
TP	mg/dl	5.29	0.43	9	5.00	0.41	8	-	-	-	5.18	0.46	8	5.04	0.22	9
Alb	mg/dl	2.51	0.18	9	2.60	0.11	8	-	-	-	2.65	0.28	8	2.62	0.12	9
BUN	mg/dl	30.22	17.70	9	23.25	5.23	8	-	-	-	37.50	30.16	8	23.78	7.38	9
UA	mg/dl	2.78	0.83	9	3.10	1.42	8	-	-	-	2.58	1.15	8	3.18	2.44	9
Bili	mg/dl	0.00	0.00	9	0.00	0.0	8	-	-	-	0.00	0.00	8	4.02	0.07	9
Creat	mg/dl	0.44	0.22	9	0.28	0.18	8	-	-	-	0.38	0.47	8	0.38	0.07	9
Na	meq/l	147.33	2.65	9	148.75	2.61	8	-	-	-	145.00	2.70	8	148.67	4.0	9
K	meq/l	6.62	1.96	9	5.30	1.76	8	-	-	-	6.81	2.03	8	5.98	1.27	9
Cl	meq/l	94.44	3.13	9	92.00	4.00	8	-	-	-	90.00	5.45	8	94.00	7.28	9
CO_2	meq/l	23.33	5.29	9	26.25	1.67	8	-	-	-	23.00	4.41	8	25.78	3.80	9
Ca	mg/dl	8.80	0.37	9	8.80	0.15	8	-	-	-	8.82	0.48	8	9.00	0.32	9
P	mg/cl	6.51	2.06	9	7.35	1.08	8	-	-	-	8.13	2.53	8	6.22	0.99	9
AP	U/l	40.22	9.77	9	46.25	8.24	8	-	-	-	44.5	9.49	8	37.78	8.03	9
LDH	U/l	320.89	171.05	9	305.00	167.83	8	-	-	-	341.50	165.42	8	280.00	178.54	9
SGOT	U/l	111.78	38.14	9	119.50	32.84	8	-	-	-	143.25	83.71	8	105.56	70.06	9
SGPT	U/l	63.33	28.34	9	52.25	31.79	8	-	-	-	61.25	47.18	8	37.11	23.52	9
CPK	U/l	155.11	58.40	9	196.75	113.82	8	-	-	-	401.50	494.44	8	446.22	718.47	9

TABLE 37. TISSUE WEIGHTS FOR STUDY IV MALES

		Group A			Group B			Group C			Group D			Group E		
	Units	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N	M	S.D.	N
Heart	mg	248.89	24.72	9	213.33*	38.73	9	-	-	-	210.00*	43.21	10	215.00*	36.29	10
Lungs	mg	245.56	35.75	9	269.78	43.31	9	-	-	-	239.00	55.67	10	225.00	29.16	10
Spleen	mg	284.37	113.92	9	275.51	89.24	9	-	-	-	237.91	92.37	10	241.51	97.47	10
Liver	mg	2051.25	265.41	9	1861.11	231.97	9	-	-	-	1821.00	214.09	10	1982.50	126.30	10
Kidney	mg	685.56	92.48	9	625.56	116.09	9	-	-	-	616.00	119.55	10	609.00	89.37	10
Adrenals	mg	14.10	3.19	9	14.22	4.89	9	-	-	-	12.46	1.92	10	10.73	3.81	10
Brain	mg	460.00	24.50	9	418.89	83.13	9	-	-	-	416.00*	29.14	10	431.00*	27.26	10
Testes	mg	218.89	20.20	9	218.89	40.76	9	-	-	-	225.00	41.97	10	212.22	23.86	10
Body Weight	g	37.79	5.31	9	36.13	2.30	9	-	-	-	35.81	3.44	9	37.50	2.88	10
R. Heart	10^{-3}	6.70	1.08	9	5.71	0.91	9	-	-	-	5.84	1.03	9	5.72*	0.74	10
R. Lungs	10^{-3}	6.58	1.18	9	7.48	1.29	9	-	-	-	6.51	1.15	9	6.01	0.68	10
R. Spleen	10^{-3}	7.62	3.23	9	7.58	2.69	9	-	-	-	6.36	2.33	9	6.53	2.68	10
R. Liver	10^{-3}	54.07	7.39	8	51.74	7.21	8	-	-	-	51.09	3.46	9	53.01	3.35	10
R. Kidney	10^{-3}	18.31	2.53	9	17.33	3.54	8	-	-	-	17.40	2.59	9	16.26	2.22	10
R. Adrenals	10^{-3}	0.37	0.07	9	0.38	0.13	8	-	-	-	0.34	0.04	9	0.29*	0.10	10
R. Brain	10^{-3}	12.38	1.74	9	11.48	2.54	8	-	-	-	11.66	0.83	9	11.53	0.73	10
R. Testes	10^{-3}	5.88	0.81	9	5.93	1.12	9	-	-	-	6.19	0.87	9	5.68	0.78	10

TABLE 38. DEGREE OF SIGNIFICANCE OF BLOOD CHEMISTRY RESULTS FOR STUDY I MALES

		Group A		Group B			Group C			Group D			Group E		
	Units	Min.	Max.	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P
Glu	mg/dl	80.0	398.0	128.0	358.0	.224	108.0	350.0	.869	98.0	395.0	.900	100.0	358.0	.623
Chol	mg/dl	110.0	230.0	128.0	226.0	.511	156.0	256.0	.046	136.0	270.0	.771	168.0	258.0	.054
Trig	mg/dl	8.0	94.0	0.0	64.0	.072	8.0	96.0	.175	8.0	99.0	.487	6.0	99.0	.700
TP	g/dl	3.0	5.2	4.0	5.4	.764	4.2	5.4	.132	4.2	5.2	.476	4.4	5.0	.979
Alb	g/dl	1.6	3.0	2.2	3.0	.581	1.4	3.0	.879	2.2	2.8	.830	2.2	3.0	.902
BUN	mg/dl	10.0	40.0	14.0	32.0	.359	12.0	34.0	.177	14.0	32.0	.344	14.0	30.0	.061
UA	mg/dl	0.00	8.2	0.8	7.8	.333	0.4	7.8	.512	1.2	5.0	.196	1.0	5.8	.034
Bili	mg/dl	0.0	0.2	0.0	9.0	.307	0.0	0.2	.739	0.0	0.2	.928	0.0	0.2	.928
Creat	mg/dl	0.0	1.0	0.2	0.6	.261	0.0	0.8	.136	0.2	1.4	.000	0.2	7.2	.374
Na	meq/l	88.0	150.0	134.0	150.0	.151	136.0	148.0	.150	138.0	158.0	.166	136.0	156.0	.008
K	meq/l	3.2	9.2	4.2	8.4	.304	3.2	7.8	.043	4.2	9.2	.005	4.0	7.6	.256
Cl	meq/l	28.0	98.0	82.0	126.0	.070	42.0	102.0	.307	80.0	116.0	.840	78.0	104.0	.006
CO_2	meq/l	8.0	24.0	12.0	28.0	.000	12.0	24.0	.090	16.0	28.0	.008	16.0	26.0	.011
Ca	mg/dl	5.4	9.4	8.0	10.2	.041	5.2	9.4	.458	8.0	9.4	.095	7.8	9.2	.437
P	mg/dl	4.2	9.8	4.6	9.4	.191	5.0	9.8	.961	4.4	11.0	.500	5.2	9.4	.689
AP	U/l	44.0	116.0	50.0	98.0	.284	44.0	649.0	.415	56.0	168.0	.229	154.0	102.0	.217
LDH	U/l	126.0	696.0	154.0	850.0	.241	0.00	822.0	.593	122.0	738.0	.701	144.0	662.0	.621
SGOT	U/l	30.0	218.0	3.0	228.0	.224	38.0	228.0	.542	36.0	210.0	.441	38.0	272.0	.776
SGPT	U/l	2.0	144.0	6.0	124.0	.307	0.0	198.0	.707	6.0	104.0	.117	6.0	200.0	.698
CPK	U/l	32.0	1488.0	68.0	2000.0	.610	82.0	1242.0	.609	50.0	1088.0	.572	22.0	2002.0	.638

TABLE 39. DEGREE OF SIGNIFICANCE OF TISSUE WEIGHTS FOR STUDY I MALES

		Group A		Group B			Group C			Group D			Group E		
	Units	Min.	Max.	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P
Heart	mg	156.3	266.5	32.1	245.6	.441	149.8	260.0	.381	149.5	240.0	.930	123.4	249.4	.842
Lungs	mg	160.1	267.5	39.7	340.0	.115	160.3	324.5	.464	158.2	483.3	.147	138.0	420.0	.227
Spleen	mg	39.6	800.0	55.9	436.0	.593	16.1	175.4	.350	50.9	325.4	.326	15.1	103.5	.202
Liver	mg	1008.0	1940.0	1240.0	2040.0	.217	1070.0	1970.0	.816	1060.0	2290.0	.137	1220.0	2120.0	.202
Kidney	mg	440.0	680.0	340.0	750.0	.217	370.0	660.0	.392	440.0	740.0	.293	120.0	790.0	.160
Adrenals	mg	2.7	12.6	1.1	9.0	.861	1.3	92.7	.576	1.3	8.7	.089	1.7	10.3	.792
Brain	mg	304.0	460.0	330.0	490.0	.177	330.0	460.0	.217	380.0	490.0	.028	370.0	480.0	.003
Testes	mg	190.0	286.2	103.8	301.3	.985	180.0	296.2	.792	196.3	287.6	.199	178.4	278.2	.384
Body Weight	g	30.0	58.0	36.0	58.0	.406	34.0	58.0	.812	33.0	56.0	.881	36.0	60.0	.545
R. Heart	10^{-3}	2.84	6.11	0.85	6.19	.098	3.40	6.05	.228	3.33	6.29	.938	2.75	5.84	.376
R. Lungs	10^{-3}	3.37	6.36	0.88	8.10	.501	3.58	8.32	.778	3.44	13.06	.295	2.94	8.58	.669
R. Spleen	10^{-3}	1.29	20.0	1.36	9.69	.508	0.31	4.62	.361	1.28	8.80	.362	0.30	2.58	.497
R. Liver	10^{-3}	21.45	57.27	26.38	44.52	.472	26.22	43.56	.729	29.32	49.78	.179	28.33	48.61	.405
R. Kidney	10^{-3}	8.73	17.11	7.56	18.16	.898	8.22	15.28	.155	9.29	18.97	.477	2.86	19.75	.524
R. Adrenals	10^{-3}	0.07	0.27	0.03	0.21	.529	0.03	2.51	.546	0.03	0.24	.090	0.05	0.20	.385
R. Brain	10^{-3}	6.36	14.00	6.35	12.90	.617	7.33	12.94	.521	6.96	14.00	.384	6.83	12.84	.360
R. Testes	10^{-3}	3.62	7.87	2.41	6.87	.333	3.91	7.21	.460	3.78	8.72	.293	3.86	6.96	.154

TABLE 40. DEGREE OF SIGNIFICANCE OF BLOOD CHEMISTRY RESULTS FOR STUDY I FEMALES

		Group A		Group B			Group C			Group D			Group E		
	Units	Min.	Max.	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P
Glu	mg/dl	46.0	292.0	36.0	312.0	.042	24.0	294.0	.780	54.0	268.0	.246	62.0	290.0	.470
Chol	mg/dl	88.0	186.0	108.0	196.0	.297	98.0	194.0	.427	88.0	290.0	.913	72.0	178.0	.568
Trig	mg/dl	2.0	54.0	0.0	34.0	.926	2.0	70.0	.297	0.0	26.0	.154	0.0	36.0	.466
TP	g/dl	2.4	5.2	4.2	5.2	.199	3.0	5.2	.225	3.6	5.4	.777	2.2	5.4	.828
Alb	g/dl	1.4	3.2	0.0	3.4	.818	2.0	3.2	.109	2.0	3.2	.641	1.2	3.4	.579
BUN	mg/dl	12.0	40.0	12.0	46.0	.087	6.0	54.0	.547	10.0	54.0	.004	14.0	52.0	.542
UA	mg/dl	0.0	7.8	1.1	7.8	.427	0.6	7.8	.650	0.0	7.6	.602	0.8	7.8	.159
Bili	mg/dl	0.0	0.2	0.0	1.8	.378	0.0	0.2	.820	0.0	0.2	.188	0.0	0.2	.255
Creat	mg/dl	0.0	0.6	0.2	0.6	.304	0.0	0.4	.712	0.2	0.4	.306	0.0	0.4	.768
Na	meq/l	132.0	168.0	134.0	155.0	.267	136.0	178.0	.533	118.0	160.0	.226	102.0	162.0	.531
K	meq/l	3.0	9.4	4.4	8.0	.587	4.2	7.8	.749	4.4	9.4	.250	3.0	8.2	.410
Cl	meq/l	6.0	108.0	40.0	110.0	.132	46.0	124.0	.070	48.0	114.0	.018	20.0	122.0	.174
CO_2	meq/l	6.0	26.0	10.0	26.0	.638	8.0	24.0	.976	10.0	26.0	.954	8.0	24.0	.999
Ca	mg/dl	4.4	9.6	8.2	9.6	.657	3.8	10.6	.993	6.8	10.0	.531	0.0	9.8	.100
P	mg/dl	4.8	14.4	2.0	10.4	.120	6.0	9.6	.583	5.0	10.8	.769	3.6	10.4	.360
AP	U/l	54.0	194.0	72.0	192.0	.362	54.0	212.0	.767	73.0	178.0	.591	72.0	182.0	.538
LDH	U/l	86.0	999.0	136.0	962.0	.845	136.0	784.0	.202	132.0	999.0	.768	112.0	999.0	.284
SGOT	U/l	20.0	648.0	42.0	598.0	.823	38.0	320.0	.320	24.0	730.0	.488	40.0	532.0	.713
SGPT	U/l	0.0	222.0	8.0	84.0	.909	4.0	98.0	.260	8.0	134.0	.812	6.0	138.0	.511
CPK	U/l	64.0	2768.0	50.0	2352.0	.949	40.0	2000.0	.389	104.0	1412.0	.268	48.0	1248.0	.086

TABLE 41. DEGREE OF SIGNIFICANCE OF TISSUE WEIGHTS FOR STUDY I FEMALES

	Units	Group A		Group B			Group C			Group D			Group E		
		Min.	Max.	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P
Heart	mg	125.9	255.1	120.0	218.5	.070	96.5	190.0	.038	99.4	232.6	.064	107.2	256.6	.297
Lungs	mg	110.9	268.6	111.5	305.2	.836	122.7	235.4	.794	95.2	304.6	.192	114.2	277.5	.838
Spleen	mg	54.4	123.5	37.6	125.7	.851	10.8	137.4	.419	35.5	114.0	.021	42.4	113.0	.008
Liver	mg	860.0	1510.0	640.0	1600.0	.542	740.0	1420.0	.674	720.0	1220.0	.001	920.0	1660.0	.778
Kidney	mg	170.0	620.0	270.0	460.0	.715	210.0	52.0	.769	250.0	450.0	.345	190.0	460.0	.184
Adrenals	mg	4.4	77.4	5.1	22.0	.274	2.8	13.0	.131	0.6	12.9	.193	5.2	16.9	.271
Brain	mg	380.0	840.0	300.0	540.0	.464	350.0	56.0	.680	320.0	500.0	.286	340.0	524.2	.364
Ovaries	mg	1.6	47.3	13.3	54.9	.614	7.5	99.2	.328	7.3	48.8	.496	9.8	60.8	.928
Body Weight	g	22.0	40.0	23.0	40.0	.565	14.0	34.0	.182	18.0	34.0	.031	17.0	32.0	.192
R. Heart	10^{-3}	4.0	9.11	3.64	7.80	.512	3.94	7.13	.291	3.43	8.95	.977	4.72	8.85	.978
R. Lungs	10^{-3}	4.27	10.33	4.29	9.25	.562	4.09	9.83	.314	3.28	11.28	.771	4.08	10.28	.447
R. Spleen	10^{-3}	2.02	4.57	1.57	4.25	.891	0.35	4.49	.681	1.65	4.75	.192	1.63	3.77	.020
R. Liver	10^{-3}	32.50	58.0	26.67	56.09	.183	32.26	57.86	.509	32.31	43.33	.105	31.88	63.85	.517
R. Kidney	10^{-3}	5.86	21.38	9.39	17.08	.953	8.00	17.93	.491	9.38	18.75	.483	6.33	18.34	.772
R. Adrenals	10^{-3}	0.14	2.86	0.18	0.85	.313	0.10	0.47	.168	0.02	0.52	.275	0.23	0.67	.343
R. Brain	10^{-3}	12.25	28.97	10.71	19.58	.851	12.33	27.86	.290	13.53	21.43	.316	10.97	23.53	.676
R. Ovaries	10^{-3}	0.24	1.58	0.46	1.91	.508	0.48	3.42	.206	0.35	1.54	.814	0.38	2.25	.810

TABLE 42. DEGREE OF SIGNIFICANCE OF BLOOD CHEMISTRY RESULTS FOR STUDY II MALES

		Group A		Group B			Group C			Group D			Group E		
	Units	Min.	Max.	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P
Glu	mg/dl	66.0	358.0	120.0	410.0	.322	104.0	390.0	.316	108.0	368.0	.599	60.0	470.0	.488
Chol	mg/dl	142.0	228.0	146.0	764.0	.157	160.0	232.0	.066	146.0	252.0	.072	142.0	222.0	.433
Trig	mg/dl	16.0	99.0	4.0	99.0	.832	18.0	74.0	.360	18.0	99.0	.365	2.0	70.0	.164
TP	g/dl	3.8	5.4	4.0	5.6	.075	4.6	5.2	.043	4.6	5.4	.048	4.6	5.2	.146
Alb	g/dl	2.4	3.4	2.6	3.6	.303	2.6	3.4	.104	2.6	3.6	.582	2.6	3.2	.451
BUN	mg/dl	14.0	34.0	14.0	36.0	.864	12.0	22.0	.000	12.0	28.0	.008	16.0	30.0	.186
UA	mg/dl	1.2	5.0	0.0	4.6	.692	0.0	3.6	.101	1.8	7.0	.141	1.4	5.0	.567
Bili	mg/dl	0.0	0.2	0.0	0.0	.154	0.0	0.2	.796	0.0	0.2	.670	0.0	0.0	.268
Creat	mg/dl	0.0	0.6	0.4	0.6	1.000	0.4	0.6	.975	0.4	0.8	.165	0.4	0.6	.346
Na	meq/l	128.0	160.0	150.0	160.0	.294	148.0	162.0	.157	144.0	159.0	.788	150.0	158.0	.378
K	meq/l	4.6	7.8	5.0	8.4	.815	3.2	7.4	.391	5.2	8.8	.065	5.8	8.0	.016
Cl	meq/l	80.0	108.0	94.0	108.0	.170	92.0	108.0	.114	40.0	106.0	.330	98.0	110.0	.252
CO_2	meq/l	14.0	24.0	14.0	22.0	.471	14.0	24.0	.272	8.0	24.0	.150	12.0	24.0	.182
Ca	mg/dl	6.8	9.6	8.2	10.4	.128	8.4	9.6	.786	8.4	10.0	.050	8.4	9.4	.796
P	mg/dl	5.8	10.2	6.0	12.8	.361	6.0	8.8	.189	5.6	13.2	.042	5.2	12.0	.517
AP	U/l	72.0	136.0	66.0	126.0	.655	72.0	100.0	.093	64.0	104.0	.138	70.0	112.0	.723
LDH	U/l	304.0	848.0	144.0	992.0	.158	250.0	944.0	.446	334.0	862.0	.779	232.0	942.0	.625
SGOT	U/l	54.0	196.0	76.0	470.0	.065	84.0	280.0	.019	72.0	206.0	.228	80.0	402.0	.077
SGPT	U/l	18.0	202.0	30.0	440.0	.144	30.0	168.0	.326	32.0	190.0	.199	38.0	292.0	.129
CPK	U/l	174.0	1316.0	126.0	896.0	.655	120.0	860.0	.829	120.0	700.0	.154	122.0	922.0	.438

TABLE 43. DEGREE OF SIGNIFICANCE OF TISSUE WEIGHTS FOR STUDY II MALES

		Group A		Group B			Group C			Group D			Group E		
	Units	Min.	Max.	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P
Heart	mg	145.6	232.1	155.3	205.7	.035	148.0	241.0	.623	138.5	233.9	.331	163.1	213.7	.413
Lungs	mg	173.8	340.6	168.2	266.2	.006	173.1	273.0	.012	157.4	266.5	.392	124.8	222.3	.004
Spleen	mg	44.6	196.5	21.3	105.1	.540	45.3	942.0	.001	47.3	107.5	.434	33.4	83.0	.035
Liver	mg	1090.0	2780.6	1060.8	2500.0	.836	1000.0	1840.0	.317	1154.5	1890.0	.884	1050.0	1883.7	.112
Kidney	mg	330.0	695.7	380.0	580.0	.587	58.6	640.0	.841	369.4	630.0	.750	131.4	706.0	.194
Adrenals	mg	1.1	10.1	0.5	7.3	.041	3.9	11.4	.085	1.7	6.8	.425	1.7	11.3	.119
Brain	mg	360.0	489.9	280.0	498.1	.378	310.0	488.9	.426	250.0	470.2	.476	300.0	622.2	.470
Testes	mg	150.0	252.5	157.0	238.2	.248	174.0	264.5	.016	175.6	265.4	.992	21.7	258.9	.374
Body Weight	g	28.0	54.0	30.0	52.0	.022	29.0	52.0	.067	28.0	47.0	.156	24.0	43.0	.053
R. Heart	10^{-3}	3.94	6.11	3.71	5.33	.000	3.94	7.09	.100	3.83	7.09	.054	4.41	7.51	.033
R. Lungs	10^{-3}	3.39	6.82	4.16	7.83	.640	3.89	7.26	.507	4.27	6.63	.538	4.47	6.28	.402
R. Spleen	10^{-3}	1.12	2.63	0.56	2.60	.543	1.50	3.02	.107	1.24	2.44	.698	1.04	3.19	.625
R. Liver	10^{-3}	29.46	51.49	30.00	48.08	.003	28.45	40.00	.000	29.11	43.73	.034	32.78	47.71	.824
R. Kidney	10^{-3}	8.92	21.08	9.32	15.63	.041	1.50	16.55	.184	9.15	17.29	.250	3.98	20.77	.811
R. Adrenals	10^{-3}	0.03	0.36	0.01	0.22	.025	0.10	0.32	.553	0.05	0.19	.246	0.05	0.44	.096
R. Brain	10^{-3}	8.65	16.49	6.36	13.53	.026	8.85	14.65	.036	6.76	16.74	.171	8.81	19.30	.237
R. Testes	10^{-3}	3.73	7.59	4.31	6.98	.597	3.56	8.02	.430	3.85	6.86	.443	0.84	8.36	.587

TABLE 44. DEGREE OF SIGNIFICANCE OF BLOOD CHEMISTRY RESULTS FOR STUDY II FEMALES

		Group A		Group B			Group C			Group D			Group E		
	Units	Min.	Max.	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P
Glu	mg/dl	50.0	432.0	42.0	278.0	.232	72.0	332.0	.246	68.0	302.0	.395	40.0	440.0	.484
Chol	mg/dl	94.0	196.0	74.0	174.0	.287	114.0	176.0	.782	112.0	154.0	.346	112.0	172.0	.921
Trig	mg/dl	20.0	72.0	14.0	99.0	.212	14.0	99.0	.579	16.0	68.0	.309	12.0	84.0	.102
TP	g/dl	4.8	5.6	2.6	5.4	.080	4.6	5.6	.463	4.2	5.4	.188	4.6	6.0	.861
Alb	g/dl	2.6	4.0	1.6	3.8	.531	2.2	3.6	.365	2.4	3.6	.061	2.6	4.0	.440
BUN	mg/dl	10.0	28.0	10.0	32.0	.821	10.0	18.0	.027	12.0	44.0	.784	10.0	44.0	.619
UA	mg/dl	1.4	5.6	0.0	6.0	.284	0.4	4.2	.308	0.6	4.8	.083	0.6	5.8	.445
Bili	mg/dl	0.0	0.2	0.0	0.2	.559	0.0	0.2	.758	0.0	0.2	.938	0.0	0.2	.938
Creat	mg/dl	0.4	0.8	0.2	0.8	.913	0.4	0.6	.530	0.4	0.8	.698	0.4	0.8	.580
Na	meq/l	146.0	164.0	74.0	162.0	.256	142.0	160.0	.104	146.0	160.0	.373	146.0	164.0	.910
K	meq/l	5.0	8.0	3.6	7.6	.731	5.0	9.8	.198	4.2	7.4	.087	4.8	8.4	.809
Cl	meq/l	64.0	110.0	24.0	112.0	.633	48.0	105.0	.711	94.0	114.0	.027	56.0	110.0	.837
CO_2	meq/l	6.0	24.0	4.0	24.0	.850	14.0	22.0	.407	10.0	24.0	.885	12.0	22.0	.865
Ca	mg/dl	8.0	9.6	4.6	9.4	.219	8.0	10.2	.713	8.0	9.8	.261	8.2	10.8	.278
P	mg/dl	6.2	11.6	4.4	13.4	.550	5.4	9.6	.104	5.4	10.6	.389	6.4	16.2	.376
AP	U/l	72.0	190.0	66.0	172.0	.588	34.0	200.0	.803	94.0	154.0	.059	66.0	170.0	.942
LDH	U/l	268.0	999.0	266.0	676.0	.460	164.0	999.0	.279	232.0	740.0	.262	168.0	828.0	.159
SGOT	U/l	92.0	648.0	102.0	420.0	.727	56.0	632.0	.668	60.0	420.0	.710	58.0	432.0	.415
SGPT	U/l	18.0	348.0	20.0	156.0	.996	20.0	262.0	.572	14.0	162.0	.805	16.0	168.0	.326
CPK	U/l	124.0	928.0	128.0	1052.0	.981	84.0	1720.0	.555	164.0	1092.0	.265	78.0	954.0	.399

TABLE 45. DEGREE OF SIGNIFICANCE OF TISSUE WEIGHTS FOR STUDY II FEMALES

	Units	Group A		Group B			Group C			Group D			Group E		
		Min.	Max.	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P
Heart	mg	117.1	193.1	106.0	185.5	.351	93.2	173.5	.668	123.3	210.3	.093	101.30	174.4	.671
Lungs	mg	129.1	241.4	150.2	242.8	.112	138.5	235.2	.886	138.0	230.8	.224	134.3	244.5	.820
Spleen	mg	35.6	103.6	47.5	106.3	.262	28.9	428.3	.332	28.0	119.6	.075	35.2	205.1	.376
Liver	mg	710.0	1420.0	680.0	1391.4	.623	580.0	1420.0	.357	667.3	1530.8	.844	769.4	1400.0	.329
Kidney	mg	200.0	590.0	130.0	389.1	.209	140.0	380.0	.065	96.6	388.6	.308	180.0	370.0	.084
Adrenals	mg	4.9	15.2	6.0	16.0	.986	4.9	11.3	.273	7.8	13.7	.069	3.0	16.4	.553
Brain	mg	350.0	494.1	250.0	579.1	.478	37.00	500.0	.628	390.0	478.5	.703	320.0	480.7	.422
Ovaries	mg	6.5	44.5	12.4	46.1	.343	7.3	35.7	.882	15.1	38.3	.069	10.3	44.1	.843
Body Weight	g	19.0	38.0	21.0	35.0	.364	14.0	32.0	.230	19.0	35.0	.458	19.0	36.0	.094
R. Heart	10^{-3}	4.15	6.95	4.56	7.37	.077	4.19	6.74	.350	4.74	7.83	.023	4.38	7.77	.021
R. Lungs	10^{-3}	5.07	8.41	6.04	9.69	.006	5.00	10.05	.174	5.52	10.41	.042	5.57	9.40	.088
R. Spleen	10^{-3}	1.39	3.70	2.03	4.19	.027	1.64	13.82	.181	1.47	4.43	.005	1.41	8.20	.085
R. Liver	10^{-3}	32.80	51.88	32.38	49.59	.685	31.43	55.0	.766	35.12	57.37	.223	37.50	56.00	.296
R. Kidney	10^{-3}	8.40	28.10	6.19	17.03	.441	6.36	16.67	.429	3.58	16.35	.698	8.18	14.92	.655
R. Adrenals	10^{-3}	0.16	0.63	0.23	0.62	.572	0.21	0.53	.832	0.29	0.60	.018	0.12	0.61	.141
R. Brain	10^{-3}	10.36	21.43	11.91	26.32	.232	10.32	31.43	.382	12.55	22.40	.335	12.14	24.48	.231
R. Ovaries	10^{-3}	0.31	1.59	0.50	1.71	.163	0.41	1.43	.535	0.55	1.62	.025	0.43	1.58	.387

TABLE 46. DEGREE OF SIGNIFICANCE OF BLOOD CHEMISTRY RESULTS FOR STUDY III MALES

		Group A		Group B			Group C			Group D			Group E		
	Units	Min.	Max.	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P
Glu	mg/dl	224.0	424.0	114.0	278.0	.001	140.0	306.0	.001	48.0	328.0	.061	66.0	318.0	.003
Chol	mg/dl	164.0	210.0	146.0	228.0	.800	162.0	228.0	.114	144.0	224.0	.954	140.0	226.0	.343
Trig	mg/dl	25.0	99.0	4.0	68.0	.051	18.0	99.0	.845	22.0	54.0	.017	10.0	68.0	.020
TP	g/dl	4.2	4.8	4.4	4.8	.400	4.4	5.0	.115	4.0	4.6	.136	4.2	4.6	.483
Alb	g/dl	2.4	2.6	2.4	2.8	.343	2.4	2.8	.196	2.2	2.6	.054	2.4	2.6	.098
BUN	mg/dl	16.0	28.0	18.0	24.0	.798	14.0	22.0	.012	14.0	30.0	.291	14.0	24.0	.101
UA	mg/dl	1.2	5.2	1.8	4.0	.872	1.6	4.4	.594	1.2	3.4	.066	1.2	3.2	.192
Bili	mg/dl	0.0	0.0	0.0	0.2	.104	0.0	0.0	1.000	0.0	0.2	.331	0.0	0.2	.305
Creat	mg/dl	0.2	0.6	0.0	0.4	.104	0.2	0.8	.584	0.2	0.4	.584	0.2	0.8	.182
Na	meq/l	140.0	148.0	136.0	144.0	.425	138.0	144.0	.041	138.0	148.0	.422	138.0	144.0	.250
K	meq/l	4.8	9.9	5.2	6.4	.231	5.0	6.0	.091	5.2	6.6	.229	4.8	6.4	.157
Cl	meq/l	84.0	96.0	80.0	96.0	.540	82.0	90.0	.019	86.0	96.0	.628	86.0	96.0	.728
CO_2	meq/l	18.0	24.0	18.0	26.0	.101	20.0	26.0	.139	14.0	28.0	.516	16.0	24.0	.943
Ca	mg/dl	6.8	9.6	7.4	8.2	.226	7.6	8.4	.343	7.4	8.0	.176	7.6	8.2	.390
P	mg/dl	5.4	12.4	5.2	8.4	.207	4.8	7.8	.113	5.8	10.0	.777	6.6	9.4	.918
AP	U/l	66.0	86.0	54.0	86.0	.134	58.0	104.0	.746	54.0	82.0	.008	54.0	82.0	.014
LDH	U/l	176.0	898.0	260.0	688.0	.373	214.0	710.0	.507	140.0	690.0	.576	238.0	766.0	.622
SGOT	U/l	44.0	146.0	62.0	122.0	.957	66.0	198.0	.480	52.0	148.0	.792	40.0	166.0	.321
SGPT	U/l	18.0	126.0	20.0	80.0	.518	12.0	174.0	.259	16.0	220.0	.553	20.0	152.0	.975
CPK	U/l	124.0	446.0	72.0	568.0	.464	158.0	764.0	.271	114.0	904.0	.406	70.0	346.0	.054

TABLE 47. DEGREE OF SIGNIFICANCE OF TISSUE WEIGHTS FOR STUDY III MALES

		Group A		Group B			Group C			Group D			Group E		
	Units	Min.	Max.	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P
Heart	mg	150.0	220.0	130.0	210.0	.190	120.0	250.0	.848	150.0	225.0	.763	150.0	230.0	.956
Lungs	mg	160.0	260.0	110.0	340.0	.669	110.0	300.0	.109	150.0	240.0	.373	130.0	250.0	.150
Spleen	mg	50.7	108.7	41.6	122.3	.529	49.7	99.0	.096	48.5	104.0	.019	56.5	112.0	.022
Liver	mg	145.0	2190.0	900.0	2170.0	.752	1120.0	1890.0	.829	960.0	1090.0	.674	1200.0	2360.0	.706
Kidney	mg	480.0	690.0	460.0	660.0	.346	410.0	750.0	.888	460.0	830.0	.596	500.0	690.0	.660
Adrenals	mg	3.7	10.5	3.2	8.2	.789	3.6	18.6	.012	3.8	19.3	.024	3.0	15.3	.158
Brain	mg	390.0	460.0	400.0	500.0	.060	350.0	490.0	.320	360.0	500.0	.402	360.0	440.0	.230
Testes	mg	200.0	220.0	150.0	290.0	.716	130.0	280.0	.548	140.0	230.0	.382	110.0	220.0	.017
Body Weight	g	38.0	48.0	27.0	56.0	.446	31.0	55.0	.971	24.0	51.0	.266	36.0	55.0	.683
R. Heart	10^{-3}	3.41	5.50	3.39	5.00	.984	3.47	4.77	.693	3.64	6.25	.155	3.47	5.00	.685
R. Lungs	10^{-3}	3.64	6.19	3.70	7.91	.924	2.39	6.00	.046	4.42	6.25	.690	3.41	6.10	.088
R. Spleen	10^{-3}	1.13	2.72	1.33	2.27	.226	1.38	2.08	.089	1.91	2.42	.002	1.35	2.55	.034
R. Liver	10^{-3}	3.82	45.63	28.67	46.88	.734	28.73	41.94	.993	28.04	41.05	.711	29.27	42.91	.759
R. Kidney	10^{-3}	11.40	15.00	11.32	18.15	.382	10.6	15.24	.959	10.89	19.17	.462	9.81	16.43	.588
R. Adrenals	10^{-3}	0.09	0.24	0.06	0.29	.536	0.10	0.37	.005	0.08	0.45	.010	0.07	0.35	.195
R. Brain	10^{-3}	8.13	11.58	7.96	14.82	.061	7.61	12.90	.836	8.63	15 00	.093	6.92	11.32	.186
R. Testes	10^{-3}	4.26	5.79	4.26	6.67	.087	2.83	6.77	.472	4.31	5.83	.416	3.06	5.37	.027

TABLE 48. DEGREE OF SIGNIFICANCE OF BLOOD CHEMISTRY RESULTS FOR STUDY III FEMALES

		Group A		Group B			Group C			Group D			Group E		
	Units	Min.	Max.	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P
Glu	mg/dl	124.0	298.0	108.0	230.0	.119	76.0	264.0	.058	86.0	234.0	.010	66.0	178.0	.002
Chol	mg/dl	126.0	198.0	108.0	184.0	.081	78.0	150.0	.004	114.0	160.0	.126	106.0	152.0	.004
Trig	mg/dl	6.0	40.0	12.0	42.0	.653	8.0	99.0	.317	12.0	34.0	.510	12.0	20.0	.095
TP	g/dl	4.2	5.4	4.2	4.8	.718	4.0	4.8	.724	4.2	5.0	.501	4.2	4.6	.778
Alb	g/dl	2.4	3.0	2.4	2.8	.311	2.4	2.8	.344	2.4	3.0	.659	2.4	2.8	.635
BUN	mg/dl	12.0	26.0	10.0	18.0	.000	8.0	34.0	.052	10.0	20.0	.002	10.0	18.0	.019
UA	mg/dl	1.0	5.0	1.4	4.8	.691	0.6	3.6	.772	2.0	4.8	.715	1.4	4.6	.685
Bili	mg/dl	0.0	0.0	0.0	0.0	1.000	0.0	0.2	.357	0.0	0.2	.332	0.0	0.0	1.000
Creat	mg/dl	0.0	0.4	0.0	0.8	.909	0.2	0.8	.024	0.2	0.6	.093	0.4	0.6	.005
Na	meq/l	138.0	146.0	140.0	146.0	.447	136.0	144.0	.048	138.0	148.0	.624	140.0	148.0	.943
K	meq/l	4.8	6.6	4.6	6.0	.171	4.6	5.8	.134	4.4	6.8	.555	5.0	9.9	.264
Cl	meq/l	82.0	92.0	82.0	102.0	.234	84.0	92.0	.486	80.0	98.0	.046	88.0	98.0	.003
CO_2	meq/l	18.0	90.0	16.0	24.0	.227	14.0	24.0	.134	14.0	24.0	.164	10.0	20.0	.117
Ca	mg/dl	8.0	9.2	7.8	8.4	.101	7.8	8.6	.285	7.4	9.0	.042	7.6	9.6	.755
P	mg/dl	3.8	7.4	3.8	8.8	.526	3.8	12.2	.900	5.2	9.4	.311	5.2	10.6	.177
AP	U/l	94.0	152.0	94.0	174.0	.056	68.0	320.0	.501	88.0	188.0	.089	82.0	114.0	.158
LDH	U/l	136.0	480.0	128.0	572.0	.761	8.2	492.0	.545	172.0	732.0	.128	202.0	594.0	.363
SGOT	U/l	46.0	342.0	52.0	284.0	.964	60.0	354.0	.695	66.0	386.0	.452	98.0	244.0	.263
SGPT	U/l	4.0	236.0	10.0	124.0	.536	22.0	174.0	.903	16.0	138.0	.922	10.0	94.0	.904
CPK	U/l	62.0	246.0	52.0	428.0	.591	88.0	862.0	.108	112.0	698.0	.081	106.0	452.0	.077

TABLE 49. DEGREE OF SIGNIFICANCE OF TISSUE WEIGHTS FOR STUDY III FEMALES

		Group A		Group B			Group C			Group D			Group E		
	Units	Min.	Max.	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P
Heart	mg	50.0	190.0	100.0	190.0	.184	90.0	230.0	.310	120.0	170.0	.286	120.0	170.0	.086
Lungs	mg	150.0	230.0	160.0	250.0	.426	110.0	260.0	.727	170.0	230.0	.141	160.0	230.0	.735
Spleen	mg	27.1	77.7	16.5	90.7	.949	37.0	128.1	.006	47.0	145.5	.088	48.6	106.0	.020
Liver	mg	900.0	1590.0	1030.0	1590.0	.874	1100.0	2270.0	.225	1200.0	1490.0	.296	1070.0	1420.0	.172
Kidney	mg	250.0	390.0	270.0	42.0	.028	250.0	500.0	.377	230.0	360.0	.449	150.0	400.0	.818
Adrenals	mg	2.7	13.0	3.4	18.4	.028	10.0	23.0	.000	3.1	33.6	.020	7.5	22.4	.042
Brain	mg	340.0	470.0	400.0	460.0	.525	370.0	490.0	.866	320.0	430.0	.064	240.0	460.0	.261
Ovaries	mg	8.9	28.7	6.9	37.6	.074	19.3	42.0	.033	10.0	39.6	.384	13.2	36.2	.053
Body Weight	g	20.0	37.0	28.0	40.0	.077	28.0	44.0	.044	24.0	34.0	.469	26.0	38.0	.724
R. Heart	10^{-3}	2.51	5.83	3.03	6.00	.739	2.14	6.57	.970	3.53	5.86	.095	4.00	6.54	.125
R. Lungs	10^{-3}	4.29	8.33	4.47	6.94	.335	2.62	7.43	.201	6.00	7.50	.145	5.14	7.69	.753
R. Spleen	10^{-3}	0.97	3.10	0.43	2.76	.490	1.32	3.75	.071	1.76	5.01	.069	1.87	3.12	.038
R. Liver	10^{-3}	38.11	56.67	33.25	46.0	.013	30.56	81.07	.762	38.23	54.17	.097	33.68	51.54	.078
R. Kidney	10^{-3}	8.11	12.50	8.18	13.33	.752	5.95	13.89	.410	6.77	14.17	.933	5.00	13.46	.925
R. Adrenals	10^{-3}	0.11	0.42	0.10	0.61	.082	0.27	0.69	.002	0.12	1.40	.026	0.20	0.75	.051
R. Brains	10^{-3}	10.83	23.00	11.05	15.71	.231	8.81	14.00	.121	9.41	17.92	.641	8.00	17.69	.354
R. Ovaries	10^{-3}	0.24	0.84	0.21	1.25	.240	0.59	1.08	.012	0.31	1.38	.245	0.51	1.25	.071

TABLE 50. DEGREE OF SIGNIFICANCE OF BLOOD CHEMISTRY RESULTS FOR STUDY IV MALES

		Group A		Group B			Group C			Group D			Group E		
	Units	Min.	Max.	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P
Glu	mg/dl	30.0	722.0	52.0	102.0	.345	-	-	-	52.0	136.0	.378	20.0	136.0	.395
Chol	mg/dl	126.0	172.0	120.0	190.0	.745	-	-	-	120.0	156.0	.641	134.0	158.0	.788
Trig	mg/dl	12.0	44.0	32.0	62.0	.159	-	-	-	24.0	42.0	.345	12.0	52.0	.793
TP	g/dl	4.6	5.8	4.6	5.8	.178	-	-	-	4.4	6.0	.603	4.6	5.2	.145
Alb	g/dl	2.2	2.8	2.4	2.8	.236	-	-	-	2.2	3.2	.232	2.4	2.8	.138
BUN	mg/dl	20.0	76.0	18.0	32.0	.302	-	-	-	16.0	99.0	.547	16.0	42.0	.328
UA	mg/dl	2.0	4.2	2.0	6.4	.572	-	-	-	1.4	5.2	.680	0.4	9.0	.648
Bili	mg/dl	0.0	0.0	0.0	0.0	1.000	-	-	-	0.0	0.0	1.000	0.0	0.2	.332
Creat	mg/dl	0.2	1.0	0.0	0.4	.106	-	-	-	0.0	1.4	.696	0.2	0.4	.394
Na	meq/l	144.0	152.0	146.0	152.0	.285	-	-	-	140.0	150.0	.152	144.0	158.0	.417
K	meq/l	4.8	9.9	3.8	8.8	.167	-	-	-	4.6	9.9	.847	4.4	8.4	.420
Cl	meq/l	90.0	100.0	84.0	96.0	.178	-	-	-	84.0	98.0	.054	86.0	110.0	.868
CO_2	meq/l	12.0	28.0	24.0	28.0	.157	-	-	-	14.0	28.0	.841	20.0	30.0	.277
Ca	mg/dl	8.2	9.2	8.6	9.0	1.000	-	-	-	8.0	9.4	.906	8.6	9.4	1.000
P	mg/dl	1.6	8.8	6.2	9.4	.320	-	-	-	6.0	13.2	.168	4.8	7.4	.710
AP	U/l	30.0	62.0	36.0	60.0	.192	-	-	-	32.0	58.0	.375	24.0	50.0	.570
LDH	U/l	140.0	718.0	146.0	558.0	.850	-	-	-	216.0	708.0	.805	158.0	724.0	.627
SGOT	U/l	48.0	154.0	76.0	168.0	.663	-	-	-	62.0	274.0	.325	54.0	286.0	.818
SGPT	U/l	20.0	118.0	26.0	106.0	.530	-	-	-	30.0	174.0	.921	4.0	90.0	.099
CPK	U/l	64.0	236.0	106.0	438.0	.349	-	-	-	74.0	1552.0	.157	26.0	2270.0	.243

TABLE 51. DEGREE OF SIGNIFICANCE OF TISSUE WEIGHTS FOR STUDY IV MALES

	Units	Group A		Group B			Group C			Group D			Group E		
		Min.	Max.	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P	Min.	Max.	P
Heart	mg	210.0	280.0	170.0	270.0	.034	-	-	-	150.0	300.0	.030	170.0	280.0	.031
Lungs	mg	200.0	310.0	210.0	350.0	.214	-	-	-	170.0	350.0	.767	180.0	270.0	.186
Spleen	mg	174.2	550.7	151.2	420.0	.857	-	-	-	137.6	413.4	.340	71.9	368.0	.389
Liver	mg	1550.0	2280.0	1680.0	2420.0	.136	-	-	-	1420.0	2150.0	.058	1830.0	2280.0	.478
Kidney	mg	520.0	810.0	510.0	900.0	.243	-	-	-	410.0	820.0	.178	420.0	750.0	.084
Adrenals	mg	10.1	21.6	8.2	23.6	.951	-	-	-	9.9	16.0	.187	4.0	15.0	.053
Brain	mg	430.0	500.0	270.0	580.0	.174	-	-	-	370.0	460.0	.003	400.0	480.0	.027
Testes	mg	200.0	260.0	160.0	280.0	1.000	-	-	-	140.0	290.0	.691	160.0	240.0	.532
Body Weight	g	33.3	49.0	34.0	40.0	.429	-	-	-	29.0	40.0	.384	33.0	44.0	.887
R. Heart	10^{-3}	4.49	7.88	4.86	7.71	.061	-	-	-	4.29	7.69	.103	4.47	7.03	.033
R. Lungs	10^{-3}	5.31	8.86	6.18	10.00	.802	-	-	-	5.43	8.97	.904	4.74	6.67	.207
R. Spleen	10^{-3}	4.71	15.73	43.2	8.00	.604	-	-	-	4.55	10.60	.363	1.63	9.95	.438
R. Liver	10^{-3}	45.71	63.71	46.22	69.14	.950	-	-	-	44.87	55.43	.295	46.92	58.79	.688
R. Kidney	10^{-3}	13.47	21.32	15.00	25.71	.365	-	-	-	14.14	21.03	.665	11.35	19.74	.077
R. Adrenals	10^{-3}	0.28	0.49	0.22	0.59	.098	-	-	-	0.25	0.39	.182	0.11	0.41	.036
R. Brain	10^{-3}	8.98	14.29	7.71	16.57	.307	-	-	-	9.74	12.76	.278	10.68	12.63	.171
R. Testes	10^{-3}	4.08	6.97	4.86	7.71	.389	-	-	-	4.83	7.30	.445	4.21	6.97	.612

TABLE 52. BLOOD CHEMISTRY OF 50 MICE WEIGHING 19-30 g*
(average of 100 trials)

Parameter	
Glucose (mg/dl)	174
Albumin (g/dl)	3.4
Globulin (g/dl)	2.0
Total protein (g/dl)	4.0
CPK (IU/L)	111
Alkaline Phosphatase (IU/L)	200
LDH (IU/L)	210
SGOT (IU/L)	107
SGPT (IU/L)	82
Calcium (mg/dl)	9.3
Inorganic Phosphorus (mg/dl)	5.6
Sodium (mEq/L)	110
Potassium (mEq/L)	4.3
Chloride (mEq/L)	92
Iron (mcg/dl)	102
Cholesterol (mg/dl)	50
Triglyceride (mg/dl)	100
BUN (mg/dl)	10.5
Bilirubin (mg/dl)	0.05
Uric Acid (mg/dl)	0.6
Creatinine (mg/dl)	0.5

*Scientific Associates, Inc., 6200 S. Lindbergh, St. Louis, Mo. 63123

comparison can be made. Average values were close for some parameters, such as glucose, total protein, calcium, potassium, chloride, bilirubin, and creatinine. Average values for other parameters were quite different. For example, our mean value for cholesterol in the control group is 184.4 mg/dl, and the Scientific Associates value is 50 mg/dl. The data for triglycerides show the opposite: our average is 35.5 mg/dl and the Scientific Associates value, 100 mg/dl. CPK and LDH values measured in our laboratory were 3 times the values measured in the other laboratory. Our values for these enzymes are probably higher (and show the highest variability) as a result of the blood drawing method (heart puncture). Strain differences might also account for some of the variation between the two laboratories. A more extensive data base is essential before conclusions are drawn from such laboratory studies.

Liver Mixed Function Oxidase Activity--

The mixed function oxidase (MFO) system is located in the microsomes of a number of tissues and metabolizes foreign compounds and certain endogenous derivatives. This enzymatic complex was once believed to act as a detoxifying mechanism and in the last decade was found to be an activation system for a large number of xenobiotics to their toxic derivatives. A few hundred compounds are known to induce or increase the activity of the MFO, and a smaller number of environmental pollutants are known to reduce or inhibit the system. Among the inducers, some groupings are based on chemical structure, such as the polyaromatic hydrocarbons (methylcholanthrene) or the chloro-organic compounds (DDT) and many other unrelated comopounds (such as phenobarbital). Inorganic or organic compounds, such as lead and cannabinol compounds, can inhibit the MFO activity. The activity of this system can be evaluated under in vitro or in vivo conditions. We chose to test the activity in vivo by the determination of the sleeping times induced by hexobarbital. Longer sleeping times will result from inhibition of the MFO (slower metabolism of hexabarbital), and shorter sleeping times from induction of the mixed function oxidases. Mice were injected with 100 mg/kg hexobarbital at certain times of the day, and the duration of loss of righting reflex was measured. A sample of animals, chosen using the table of random digits, was used for this test.

The results of Study I are given in Table 53 and those of Study II in Table 54. Latent time is the time between injection and the loss of righting reflex, and sleeping time is the time between loss and gain of the righting reflex. Positive controls run on mice exposed to 500 mg/kg body weight of Aroclor 1254 showed about a 50 percent shortening of sleeping time induced by 100 mg/kg hexobarbital.

The variability of the results among the mice was quite large; however, the only significant difference in relation to the controls was found in Study I, where females in Group E (exposed to the highest TOC concentration) had about 50 percent shorter sleeping time than the control. It should be kept in mind that during the long exposure time, other factors in the environment, including chemicals in food, water and air, could interfere with these studies.

TABLE 53. SLEEPING TIMES IN MICE AFTER EXPOSURE TO CONCENTRATED RECYCLED WATER (Study I)

Group	Sex	N	Latent Time (min)		Sleeping Time (min)	
			M	SD	M	SD
A	Female	18	2.47	0.69	59.30	35.68
A	Male	18	3.85	2.57	34.28	27.3
B	Female	19	4.12	5.17	48.49	23.29
B	Male	19	3.74	1.59	40.80	17.78
C	Female	20	2.13	0.79	49.95	18.26
C	Male	19	3.29	0.91	31.65	20.01
D	Female	19	2.51	1.07	56.11	23.34
D	Male	19	4.57	2.40	41.57	22.95
E	Female	20	2.59	0.81	31.91	20.90
E	Male	20	4.39	2.66	31.61	22.29

TABLE 54. SLEEPING TIMES IN MICE AFTER EXPOSURE TO CONCENTRATED RECYCLED WATER (Study II)

Group	Sex	N	Latent Time (min)		Sleeping Time (min)	
			M	SD	M	SD
A	Male	10	2.516	0.578	40.55	15.210
A	Female	10	1.966	0.690	42.508	17.800
B	Male	10	2.666	0.594	28.191	13.487
B	Female	10	2.416	0.574	34.606	14.723
C	Male	9*	2.379	0.406	42.398	14.034
C	Female	10	2.125	0.364	38.108	18.285
D	Male	10	3.740	1.974	33.791	25.970
D	Female	10	2.216	0.364	40.875	15.227
E	Male	9**	3.190	1.820	30.944	17.449
E	Female	10	2.358	0.547	40.316	8.509

*N172 did not lose righting reflex
**N243 did not lose righting reflex

Motor Activity Test--

The well-known neurological and behavioral effects of wide-spread pollutants such as pesticides and heavy metals bring into question the possibility of subtle effects in those tissues caused by long, low-level exposure to the toxins. Other areas of concern are: (1) the possible interaction of several pollutants at low, submarginal level which increases pathological effects and (2) exposure of especially sensitive groups in the population, such as fetuses, newborns or the aged.

Behavioral studies have numerous drawbacks. In general, such studies are expensive, long-term and difficult to interpret. Therefore, a short, simple test was selected as an explorative study. The activity test measures the number of movements per arbitrary time period. The measurement is based on the interruption of a light beam which is sensed by a photoelectric cell and translated to counts. Results of this test are presented in Tables 55 and 56. There were no significant differences between any one of the experimental groups and the control.

Reproduction--

The reproduction test was performed in Study P and Study III. Both parents were exposed to concentrated water. The main difference between these studies was that in Study III the mice were exposed throughout gestation and lactation, while in Study P the mice were exposed from the age of five weeks. In both studies, the offspring were exposed throughout gestation.

There were 10 males and 20 females per group. Births started 21 days after mating and ended by the 27th day. Analysis of the weights of the newborns showed that both birth weight and weight gain are significantly dependent on the size of the litter. A regression line was computed for the relationship between litter size and mean body weight in the two experiments. The coefficients derived from these lines are presented in Table 57.

TABLE 57. DEPENDENCE BETWEEN LITTER SIZE AND MEAN BODY WEIGHT

Study	N	Correlation Coefficient (r)	Slope	Intercept	Total Mean Body Weight (g)
P	82	-0.82	-1.49	16.66	9.42
III	43	-0.80	-1.43	22.89	14.77

The results show that the correlation is negative (the higher the litter size, the lower the mean body weight) and highly significant. Mean body weights and litter size for the second experiment (study III) are given in Table 58. Similar results were found in the first experiment (study P). The corrected mean body weights (related to the litter size) at day 28 for the two experiments are given in Table 59.

TABLE 55. MOTOR ACTIVITY IN MICE AFTER EXPOSURE TO CONCENTRATE RECYCLED WATER (Study I)

Group	Sex	N	Count per First Five Minutes		Count per Ten Minutes	
			M	SD	M	SD
A	Female	16	118.75	33.54	216.68	67.25
A	Male	18	106.61	44.33	190.38	68.78
B	Female	14	133.79	64.79	249.86	118.53
B	Male	14	137.89	68.53	245.86	67.14
C	Female	17	152.88	109.95	288.82	207.37
C	Male	14	104.00	22.22	194.35	43.54
D	Female	15	126.20	29.30	250.73	56.62
D	Male	15	110.20	41.77	208.00	71.53
E	Female	14	133.57	72.15	251.81	115.10
E	Male	15	125.14	25.42	226.93	60.50

TABLE 56. MOTOR ACTIVITY IN MICE AFTER EXPOSURE TO CONCENTRATE RECYCLED WATER (Study II)

Group	Sex	N	Count per First Five Minutes		Count per Ten Minutes	
			M	SD	M	SD
A	Male	10	112.6	33.0	194.3	52.0
A	Female	10	117.4	51.8	205.4	85.2
B	Male	10	126.6	55.1	224.6	88.3
B	Female	10	101.8	30.1	203.9	54.4
C	Male	10	98.6	31.2	187.6	51.0
C	Female	10	117.8	16.8	213.5	28.6
D	Male	10	108.0	30.2	199.2	52.0
D	Female	10	138.9	10.1	267.6	131.4
E	Male	10	96.7	24.6	201.7	50.5
E	Female	10	132.5	59.9	247.2	115.8

TABLE 58. LITTER SIZE AND MEAN BODY WEIGHTS OF OFFSPRING BORN TO EXPOSED DAMS (Uncorrected)
(Study III)

Day	0		4		7		11	
Group	N	MBW	N	MBW	N	MBW	N	MBW
A	10.8 ± 1.4	1.5 ± 0.1	7.8 ± 0.4	2.6 ± 0.5	6.4 ± 1.5	3.7 ± 0.8	5.9 ± 1.9	6.1 ± 1.5
B	11.2 ± 2.1	1.5 ± 0.1	7.9 ± 0.3	2.8 ± 0.4	7.2 ± 0.8	4.1 ± 0.4	6.8 ± 1.1	5.2 ± 0.8
C	10.9 ± 2.9	1.6 ± 0.3	5.9 ± 2.4	2.7 ± 0.8	4.6 ± 2.0	3.9 ± 1.3	4.1 ± 2.3	6.8 ± 0.8
D	10.7 ± 2.1	1.5 ± 0.1	7.3 ± 1.3	2.4 ± 0.4	6.6 ± 1.5	4.1 ± 0.5	6.1 ± 2.3	5.4 ± 0.6
E	8.9 ± 2.7	1.7 ± 0.3	6.7 ± 1.5	2.7 ± 0.3	5.9 ± 1.5	4.0 ± 0.7	5.1 ± 1.3	5.8 ± 1.0

-continued-

Day	21		28	
Group	N	MBW	N	MBW
A	5.8 ± 1.9	9.9 ± 2.0	5.8 ± 1.9	15.4 ± 3.5
B	6.7 ± 1.2	9.3 ± 1.1	6.7 ± 1.2	13.8 ± 1.3
C	4.1 ± 2.3	12.1 ± 2.4	4.1 ± 2.3	18.2 ± 5.2
D	6.1 ± 2.3	9.2 ± 1.6	6.1 ± 2.3	13.3 ± 2.0
E	5.0 ± 1.2	10.4 ± 1.3	4.9 ± 1.1	14.5 ± 3.5

TABLE 59. CORRECTED MEAN BODY WEIGHT (Day 28)

	Group	Mean Body Weight			SD
		Measured	Calculated	Corrected	
Study P	A	10.1	10.4	9.1	3.9
	B	11.2	10.4	10.2	5.6
	C	9.2	9.4	9.2	4.0
	D	8.8	9.1	9.1	4.6
	E	7.8	8.0	9.2	3.1
Study III	A	15.4	14.3	15.9	3.5
	B	13.8	13.2	15.4	1.3
	C	18.2	16.3	16.7	5.2
	D	13.3	13.9	14.2	2.0
	E	14.5	15.4	13.9	3.5

Mean body weight was corrected as follows. Calculated mean body weight was found on the regression line at the corresponding mean litter size for each group. The difference between the mean found in the data and that computed from the regression line (the residue) was added to the total mean body weight (9.4 in the first experiment and 14.8 in the second; see Table 57). Standard deviations from the corrected mean body weights were computed from the individual differences between the mean body weight of each litter and its calculated number. These values were found to be very close to the original standard deviations. No significant differences were found among the different groups with relation to litter size or body weight. Measurement taken on the pregnant mothers also showed no difference in the mean of body weights or food consumption in the various experimental groups (Table 60).

TABLE 60. BODY WEIGHTS AND FOOD CONSUMPTION OF PREGNANT FEMALES

Group	Number	Body Weight (g)		Food Consumption (g)	
		$\bar{x}$	S.D.	$\bar{x}$	S.D.
A	20	29.0	3.5	8.4	1.4
B	20	30.7	3.2	8.2	1.5
C	20	31.9	2.9	8.2	1.3
D	20	31.9	3.8	8.2	1.4
E	20	29.3	3.1	8.4	1.5

Dominant Lethal Mutation Test--

The dominant lethal mutation test has been used extensively to assess the mutagenicity of a wide variety of substances. The standard method has been to give adult male animals the chemical by a certain route and schedule followed by mating each male weekly with two virgin females for 5-8 weeks. Exposed females were mated with unexposed males for one week and sacrified after another week (on day 15).

The test was run on two animal groups. The first group was 50 male mice exposed for 14 days to concentrated, recycled water. The second group consisted of 50 male and 50 female mice exposed for 90 days (since weaning). Each group was subdivided into 5 groups: A (control) and E, D, C, and B, exposed to undiluted concentrated renovated water (700 mg/l TOC) and dilutions of 1:2, 1:4, and 1:8, respectively. The summary of the study is given in Table 61 and the results of experiments I, II and III are given in Tables 62, 63, and 64.

TABLE 61. RESEARCH PLAN

Experiment	Exposure Time (days)	Sex	Significance of the Effect: Number of Dead Fetuses	Significance of the Effect: Percentage of Dead Fetuses
I	14	Male	$p<0.05$	$p<0.05$
II	90	Male	$p>0.3$	$p>0.3$
III	90	Female	$p=0.07$	$p=0.07$

In the first experiment (exposure time = 14 days), the mean number of dead fetuses per pregnant female in all groups was double the number in the control. This was found to be significant ($p<0.05$). In the second experiment (exposure time = 90 days), there was no significant difference between the groups. Although the mean number of fetuses in the experimental group was higher in the second experiment than in the first, the control also increased as much as twice; therefore, the difference was insignificant. In the third study, females (exposed for 90 days) were mated with virgin males and sacrificed after 14 days. Here, again, the mean number of dead fetuses was more than double that in the control group, but because of the relatively small number of animals, the p value (0.07) was only close to the significance border line.

Pathology--

All animals from the first 90-day study were subjected to a complete post-mortem examination. Microscopic slides prepared from groups A and E included the following tissues: heart, lungs, kidney, spleen, liver, brain, testes, ovaries, uterus, and mandibular lymph nodes. These slides were examined histologically.

TABLE 62. EFFECT OF ADMINISTRATION OF CONCENTRATED RENOVATED WATER ON SURVIVAL OF FETUSES
(Study I)

Group	Number of Females		Percentage	Number of Fetuses			Percent of Fetuses		Mean Number of Fetuses per Pregnant Female		
	Pregnant	Non-Pregnant	of Pregnancy	Total	Live	Dead	Live	Dead	Total	Dead	Live
A	84	16	84	926	889	37	96.0	4.0	11.0	0.4	10.6
B	81	18	82	913	853	60	93.4	6.6	11.3	0.8	10.5
C	76	22	78	891	830	61	93.2	6.8	11.7	0.8	10.4
D	63	35	64	686	633	53	92.2	7.8	10.9	0.9	10.0
E	65	24	73	678	632	46	93.2	6.8	10.4	0.7	9.7

TABLE 63. EFFECT OF ADMINISTRATION OF CONCENTRATED RENOVATED WATER ON SURVIVAL OF FETUSES
(Study II)

Group	Number of Females		Percentage	Number of Fetuses			Percent of Fetuses		Mean Number of Fetuses per Pregnant Female		
	Pregnant	Non-Pregnant	of Pregnancy	Total	Live	Dead	Live	Dead	Total	Dead	Live
A	71	26	73	855	797	58	93.2	6.8	12.0	0.8	11.2
B	72	25	74	903	830	73	91.9	8.1	12.5	1.0	11.5
C	81	19	81	988	906	82	91.7	8.3	12.2	1.0	11.0
D	80	20	80	997	918	79	92.0	7.9	12.5	1.0	11.5
E	76	24	76	993	848	85	90.9	9.1	12.3	1.1	11.2

TABLE 64. EFFECT OF ADMINISTRATION OF CONCENTRATED RENOVATED WATER ON SURVIVAL OF FETUSES (Study III)

Group	Number of Females		Percentage of Pregnancy	Number of Fetuses			Percent of Fetuses		Mean Number of Fetuses per Pregnant Female		
	Pregnant	Non-Pregnant		Total	Live	Dead	Live	Dead	Total	Dead	Live
A	7	3	70	86	82	4	95.3	4.7	12.3	0.6	11.7
B	6	4	60	65	59	6	90.8	9.2	10.8	1.0	9.8
C	5	5	50	65	60	5	92.3	7.7	13.0	1.0	12.0
D	5	5	50	58	48	10	82.8	17.2	11.6	2.0	9.6
E	5	5	50	69	61	8	88.4	11.6	13.8	1.6	12.2

The only gross abnormality observed at post-mortem was an infrequent pale tan liver. Microscopic study of groups A and E revealed the tissues to be within normal limits with the exception of some mild, non-specific, reversible degenerative liver change occurring with a near equal frequency in the male mice of both groups. Also observed were three benign lung neoplasms (alveolar cell adenoma), a spontaneous lesion not uncommon in the mouse lung.

The same protocol was followed for the second 90-day study, but only half as many animals were involved. No microscopic abnormalities were observed, with the exception of a small number of the mild degenerative liver change as seen in the first study. The pathology findings in both 90-day studies are insignificant and reveal only the spontaneous changes expected in a mouse population of similar size and age.

In Vitro Studies

Recycled water was found to be mutagenic in the in vivo test (dominant lethal mutation test) in the study reported here and in a previous study (5). A microbiological test using Salmonella strains and the mamalian mutagenicity assays were instituted to verify the results of the dominant lethal mutation test.

A related problem, the question of carcinogenicity, is even more complex. Conventional carcinogenicity bioassays require prohibitive volumes of water. Therefore, short in vitro assays, which demand only a small amount of sample, were used to evaluate the potential carcinogenicity of the reused water. Such tests are based on the following assumptions:

(1) Mutation is one of the first steps leading to the formation of a tumor, and certain cellular changes (transformation) in vitro are indicators of a progressive process leading to malignancy. Recent experimental work has supported these assumptions. Several hundred compounds (known carcinogens and noncarcinogens) were shown to give a very high correlation in a bacterial mutagenicity test.
(2) There is a progression of changes with time transforming "normal cells" to malignant cells.
(3) There is a relation between transformation and mutagenicity in the same cell system.
(4) There are cases when chemically induced transformed cells (in vitro) are capable of forming tumors in animals.
(5) Promotors can increase the rate of transformation or mutation induced by chemicals in tissue culture systems.

Salmonella/Microsome Test--

The Salmonella/microsome test, developed by Ames and coworkers (12), measures the reversion rate of several specially constructed Salmonella strains unable to grow in the absence of histidine. Besides the histidine mutation, the strains have undergone a mutation which causes loss of the excision repair system and a mutation which results in loss of the lipopolysaccharide coating of the bacteria. These mutations render the bacteria

more susceptible to mutagenesis. Strains TA1538, TA98, and TA1547 have frame-shift mutations in the histidine operon, and strains TA100 and TA1535 have base-pair substitutions. Since many substances are not mutagenic (or carcinogenic) prior to metabolic conversion, isolated mammalian liver microsomes were included in the test systems to provide activation. The results presented in Table 65 show that the water did not induce significant mutation (i.e., 32 x control) in the bacteria. Additional attempts (not shown) using strains 1538, 1557, and 1535 did not reveal any increase in the mutation rates.

Mutagenicity Test with Mammalian Cells--

Mutagenicity tests using mammalian cells differ from those using bacteria in the gene structure and organization, in the enzyme repair systems, and in the transport mechanisms of compounds through membranes. In addition, the Salmonella test applies a backward mutation assay while the mammalian test examines forward mutation. The same rat liver fraction was used for activation in both assays.

A problem was encountered in the initial experiments with direct mutagenicity. The cells that were exposed to the water samples still covered the plates at the end of the experiments. It appeared that the inhibitor ouabain was not as effective as in the controls. Although no further studies were carried out to explain this phenomenon, it is probable that a competitive inhibitor, such as potassium, which is present in this water at a high concentration, could interfere with the action of ouabain. Such interferences should be taken into account when testing environmental samples which contain a variety of soluble materials. Two modifications were made to solve the problem. First, the cells were trypsinized after exposure and plated in new plates; second, the concentration of ouabain was raised to 5 mM.

Table 66 shows the results of the mutagenicity test without activation, and Tables 67 and 68 present the results of two different runs after activation with rat liver fraction. While the results without activation show a marginal effect, if any, those after activation show a clear mutagenic effect in a dose-response relationship. Comparison of the plating efficiency in Tables 67 and 68 shows that toxicity is increased substantially by the liver activation system. This phenomenon is not specific to V79 cells and was studied in more detail with human fibroblasts (WI38, see p. 85).

Soft Agar Transformation Assay--

Evidence derived from <u>in vitro</u> carcinogenic experiments indicates that a progressive process leads to the development of a malignant cell (17). One of the early noted changes is a morphological transformation, while at a later stage the ability to grow on soft agar (anchorage independence) is manifested. This anchorage independance is believed to be highly correlated with cell malignancy (18). Based on the assumption that cellular transformation has a lag period and increases with time, the test was performed repeatedly for an extended time after treatment.

TABLE 65. SALMONELLA MUTAGENICITY TEST OF CONCENTRATED REUSED WATER*

	S_9	Number of Colonies per Plate TA98	TA100
Control	-	32, 37	140, 155
Control	+	55, 57	226, 236
0.1 ml H_2O	-	58, 45	216, 236
0.5 ml H_2O	-	56, 46	216, 230
0.1 ml H_2O	+	84, 64	340, 316
0.5 ml H_2O	+	90, 78	296, 326
BP (5µg)	+	689, 761	1011, 1098

*S_9 fraction is the supernatant obtained from liver homogenate after centrifugation at 9000 g for 15 minutes. The TOC concentration in water tested was 700 µg/ml. BP - Benzo(a)pyrene.

TABLE 66. DIRECT MUTAGENICITY OF CONCENTRATED RECYCLED WATER

Sample	Average Plating Efficiency (%)	Colonies per 16 Plates	Mutants per 10^6 Survivors
Control	91.0	1	0.56
1 µg/ml MNNG[a]	48.5	138	161.8
0.1 ml H_2O[b]	85.0	3	2.0
0.2 ml H_2O	82.8	0	0
0.3 ml H_2O	77.5	2	1.5
0.4 ml H_2O	76.2	1	0.75
0.5 ml H_2O	71.2	3	2.4

[a]MNNG-N-Methyl-N-nitro-N-nitrosoguanidine

[b]The concentration of TOC in water is 700 µg/ml.

TABLE 67. MUTAGENICITY OF CONCENTRATED RECYCLED WATER (first run)[a]

Sample	Toxicity (% Plating Efficiency)	Colonies per 16 plates	Oubain-Resistant Mutants/10^6 Survivors
Control	88	1	0.5
Control + S9	78	0	0.0
0.1 ml H_2O+S9	44	1	1.0
0.2 ml H_2O+S9	46	4	3.8
0.3 ml H_2O+S9	24	6	10.9
0.5 µg BP+S9	55	54	43.8
1.0 µg BP+S9	38	104	142.2

[a] H_2O mixed 1:1 with 2 x MEM and added to each sample with 1 ml S9 mix plus PBS to give 2 ml final volume. TOC level in water was 700 µg/ml. Cells incubated with S9 alone have a plating efficiency of 70%. S9 is the rat liver homogenate supernate after centrifugation at 9000 g. BP-Benzo(a)pyrene.

TABLE 68. MUTAGENICITY OF CONCENTRATED RECYCLED WATER (second run)[a]

Sample	Toxicity (% Plating Efficiency)	Colonies per 16 plates	Oubain-Resistant Mutants/10^6 Survivors
Control	90	0	0
Control + S9	77	0	0.0
0.1 ml H_2O+S9	45	11	14.0
0.2 ml H_2O+S9	39	10	14.2
0.3 ml H_2O+S9	30	15	28.1
0.4 ml H_2O+S9	44	2	2.5
1.0 µg BP+S9	32	65	120.0

[a] H_2O mixed 1:1 with 2 x MEM and added to each sample with 1 ml S9 mix plus PBS to give 2 ml final volume. TOC level in water was 700 µg/ml. Cells incubated with S9 only have a plating efficiency of 70%. S9 is the rat liver homogenate supernate after centrifugation at 9000 g. BP-Benzo(a)pyrene.

The cells were exposed for 2 hours and then assayed for growth on soft agar after 2 weeks and every 2 weeks for 2 months. Table 69 shows the results at post treatment passages 2 and 4 (PTP_2 and PTP_4). Again, while without activation there was a marginal effect, a significant increase was noted in the number of colonies on soft agar for cells incubated with liver activation system. No dose-response relationship could be shown. The spontaneous transformation increased with time as did the transformation of the exposed plates. No colonies could be detected in cells incubated with benzo(a)pyrene or MNNG up to 8 weeks after exposure.

WI38 Toxicity Test--

If reused water is approved for potable use, continuous monitoring for toxicity will probably be required. For this purpose a short, inexpensive in vitro assay using mammalian cells may be a feasible solution. Preliminary studies using human lung fibroblasts (WI38) were initiated to assess this possibility. In this assay, protein levels are used to indicate toxicity. Figure 8 shows that changes in protein level reflect cell number. Figure 9 shows that toxicity increases with time and that in the first 24 hours the effect is very small. The presence of the activation system dramatically increased the toxic effect of the water (Figure 10). Table 70 shows a dose-response relationship between the amount of water and cell protein level. Without activation, toxicity increased from 5.0 percent inhibition at 17.5 µg TOC to 24.0 percent inhibition at 210 µg TOC. In the presence of the liver fraction, the inhibition was decreased with increased TOC levels: from 87.4 percent at 17.5 µg TOC to 58.7 percent at 210 µg TOC. This phenomenon has not been explained, but may result from inhibition of the activation system by soluble chemicals in the water, or by interactions of the compounds in the water.

TABLE 69. SOFT AGAR TRANSFORMATION[a]

Sample	Average Number Colonies/Plate		Number Colonies/10^5 Cells	
	$(PTP)_2$	$(PTP)_4$	$(PTP)_2$	$(PTP)_4$
Control	6	9	4.0	6.0
0.1 ml H_2O	13	15	8.6	10.0
0.2 ml H_2O	10	17	6.6	11.3
0.2 ml H_2O + S_9	40	62	27.0	41.3
0.3 ml H_2O	11	12	7.3	8.0
0.3 ml H_2O + S_9	22	51	14.6	34.0

[a] 1.5×10^6 WI38 cells treated for 2 hr in a final volume of 2 ml. After treatment, cells were placed in a 75 cm^2 flask. Soft agar growth test 1.5×10^5 cells/dish x 10 dishes/point. TOC level in water is 700 µg/ml.

TABLE 70. THE EFFECT OF WATER CONCENTRATES ON CELL PROTEIN LEVEL IN TISSUE CULTURE

Sample	S_9	Protein (µg)	Inhibition (%)
Zero time	-	19.0	-
Untreated control	-	53.0	-
Untreated control	+	50.0	-
17.5 µg TOC	-	51.5	5.0
17.5 µg TOC	+	22.5	87.4
35.0 µg TOC	-	48.0	14.0
35.0 µg TOC	+	25.5	77.0
70.0 µg TOC	-	45.5	22.4
70.0 µg TOC	+	29.0	68.5
140.0 µg TOC	-	46.5	19.9
140.0 µg TOC	+	31.5	59.5
210.0 µg TOC	-	45.0	24.0
210.0 µg TOC	+	33.0	58.7

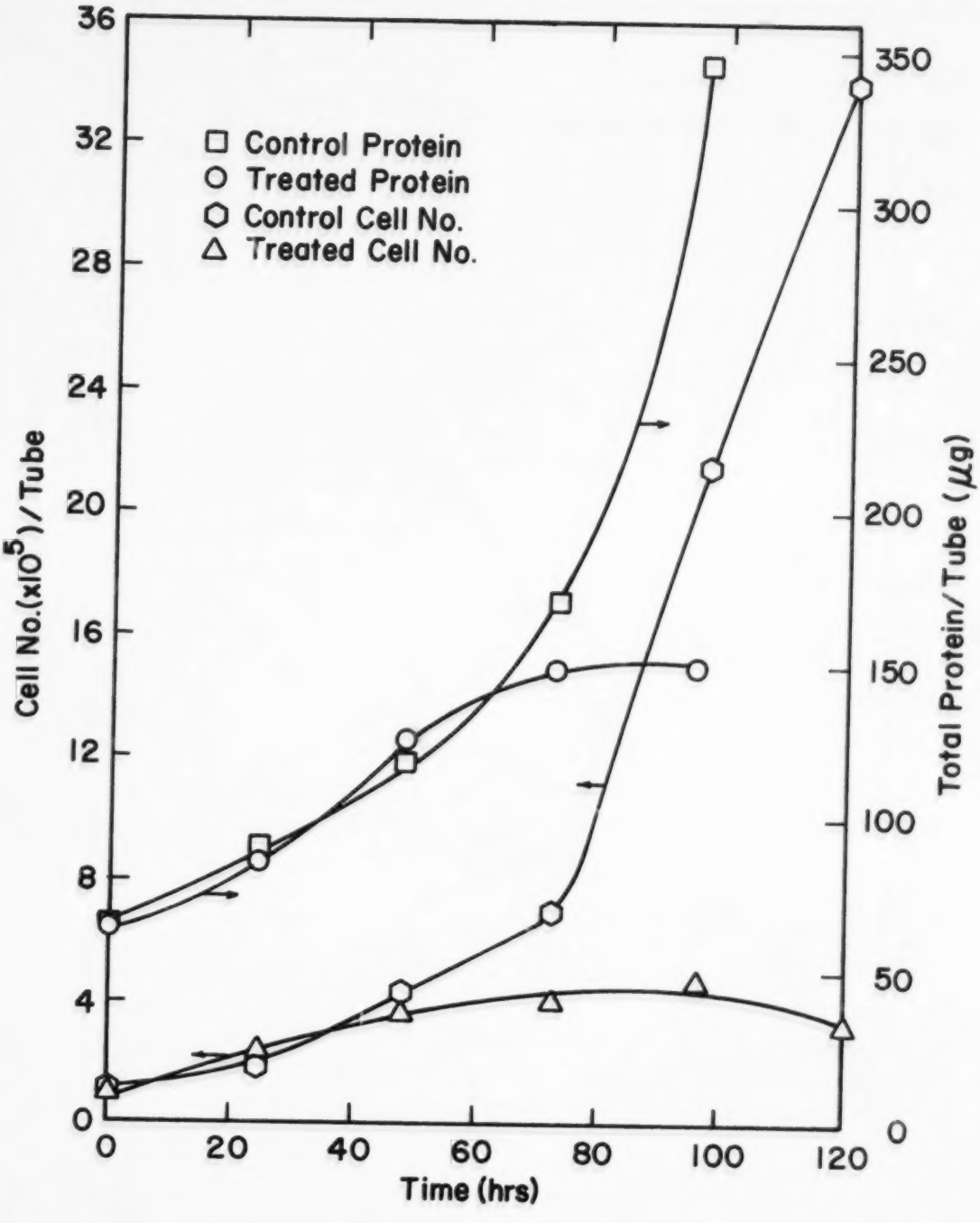


Figure 8. Cell counts and protein levels after exposure to recycled water in the presence of S9 activation system.

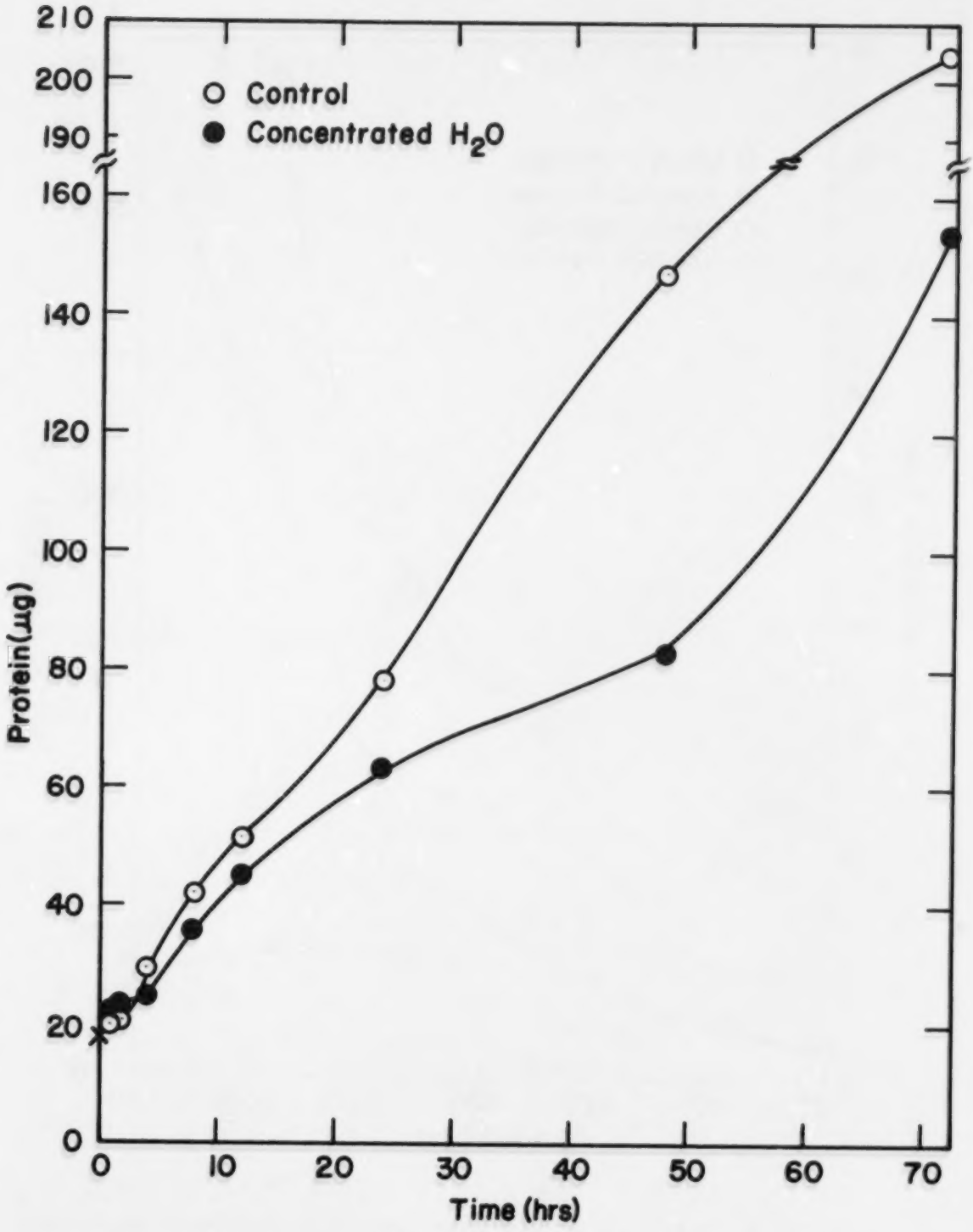


Figure 9. Direct exposure of WI38 to concentrated recycled water.

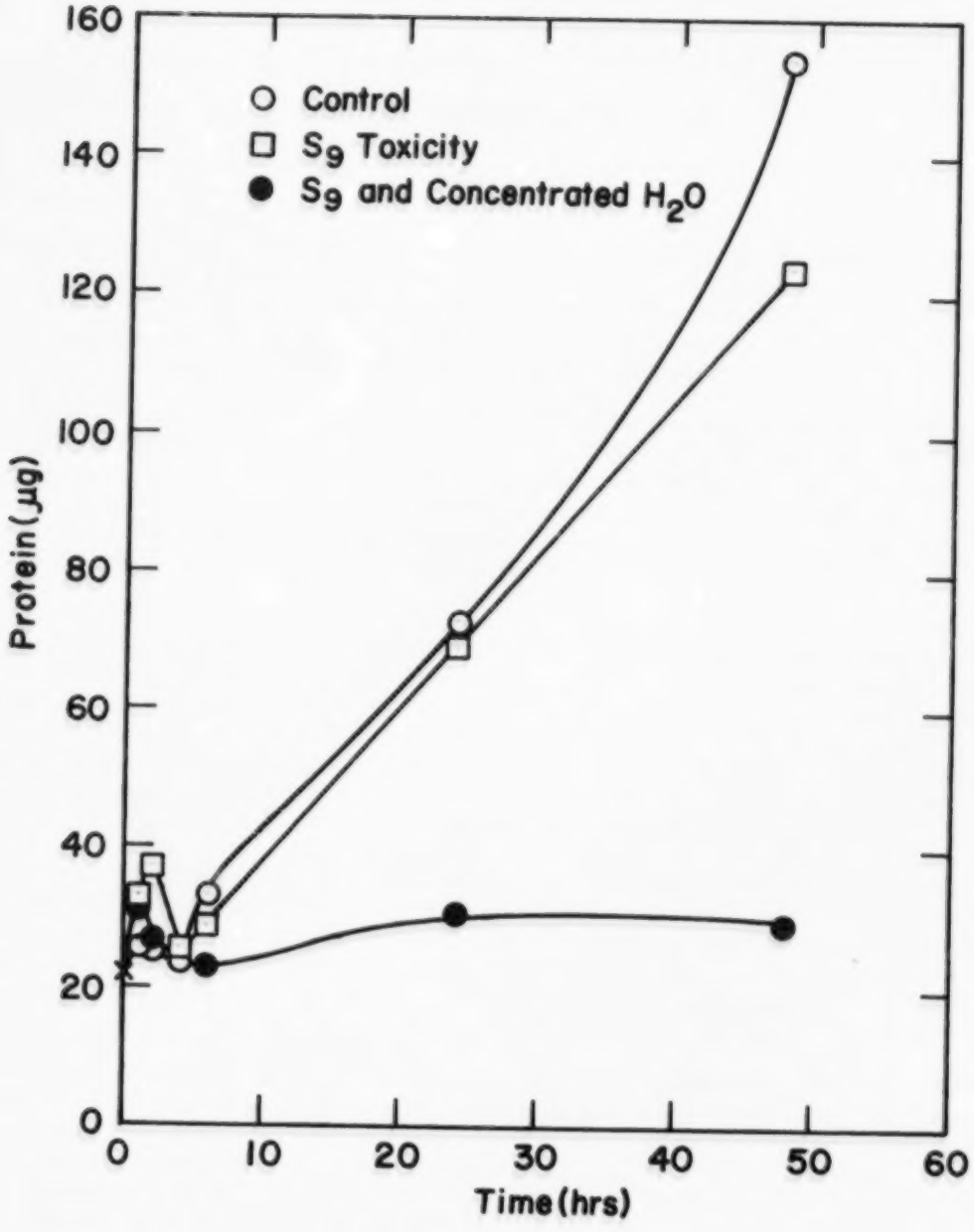


Figure 10. Effect of liver activation system on toxicity of concentrated recycled water in WI38 cells.

SECTION 5

DISCUSSION

The progress of wastewater treatment technology and increasing pressure for new sources of water have brought the subject of reuse of recycled water under intensive consideration. Direct reuse has been practiced in Southwest Africa for about 10 years (19), and long-range plans are being considered in the United States (20,21) and Israel (22).

Current wastewater treatment technology is designed to satisfy the conventional quality criteria for drinking water. However, numerous organic compounds existing in wastewater effluent cannot be removed using presently available technology. Only a limited amount of work has been done in this area, and rigorous toxicological and epidemiological studies are needed to evaluate the possible long-term effects or safety of drinking recycled water.

As a preliminary effort, this toxicological study was initiated to evaluate the health aspects of renovated water prepared in an advanced treatment plant. Three approaches were considered: (a) extensive analysis of the water followed by toxicological assessment based on data for each compound found in the water, (b) treatment of the water sample as a single test compound, or (c) a compromise approach involving the testing of a fraction of the water-soluble organic compounds.

The first approach requires and exhaustive analysis; several hundred compounds are defined in water, and these compose only a small fraction of the total organic content. In addition, toxicological data on these compounds are limited, and long-term studies will be required to evaluate the productivity of such an approach. Such an extensive study disregards interactions which could occur within the mixture (synergistic or antagonistic).

The holistic approach (water sample as one sample) has one major drawback: It is difficult to make generalized predictions for other water sources from the toxicological data obtained for a specific water sample. Based on the relative merits and disadvantages of the three approaches, the second approach was selected. A concentrated water sample was obtained with the highest possible recoveries and was used for the toxicological study.

The concentration technology selected for the study was based on reverse osmosis. Inorganic fractions were removed by techniques based on the Donnan equilibrium principle. In small-scale laboratory experiments, TOC recovery values in reverse osmosis concentrates were 80 to 90 percent (6). However, for the present work, in which large volumes (400,000 liters) were concentrated, the recovery percentage was much lower (Table 16). There is no way to determine which compounds were lost during the process. It is probable that most of the low molecular weight compounds (<150) were lost, among them the volatile weak carcinogens.

The water concentrate was incorporated into a gel type diet for feeding to the test mice. Ions contained in the concentrate were balanced according to nutritional requirements. Although the feeding practice used in this project demanded a significant effort, greater experimental control was achieved. The diet used in the study (high content of agar) was found adequate to the animals. The main characteristic of the diet was a low fiber content. The modification of toxic effects by fibers has been studied (23); however, information on this subject is limited, and the influence of this factor in this project cannot be estimated. It should be noted that any type of diet might influence the toxicity of the test sample.

Toxicological observations were made on rate of growth, food intake, fertility, mutagenicity, mortality, blood physiology and biochemistry, liver function, behavior, and general pathology. Most of the toxicological tests did not reveal any significant changes in the exposed mice. This was found true in general clinical and physiological assessment, with tests such as growth and lethality rate, and with special assays for evaluation of specific tissue fractions such as liver or the central nervous system.

The most elaborate study was an examination of blood constituents. Twenty-three parameters were checked, and only in a few cases were there significant differences between the experimental groups and the control. Although a number of significant differences were noted in two or more groups in the same study, none were repeated in other studies. No specific pathological syndrome could be related to any of the changes in the blood serum. However, it is noteworthy that the number of changes in the 150-day study was higher than in the 90-day studies. No significant changes were found in animals that were subjected to the regular diet for 90 days after an exposure time of 90 days. Although these differences are small, they might indicate that an extended exposure period beyond 150 days (about 20 percent of the animal's lifetime), might reveal more significant pathological changes.

Likewise, the pathological results did not show any significant differences among the groups. The weights of the spleen and adrenals in the various experimental groups were significantly different from the controls. Again, these differences were not accompanied by histopathological changes. The relative tissue weight analysis did not provide any increase in sensitivity compared to that with absolute tissue weights. On the contrary, more significant results were found with absolute tissue weights.

Reproduction tests did not reveal changes in fertility or in the weight or number of newborns. Results of chronic studies carried out on mice born to exposed dams were not different from those for mice exposed only after weaning (study I as compared to studies II and III). There are several possible explanations for these observations: (a) The toxic chemicals do not pass the placenta barrier or transfer through milk. (b) Doses of the toxic materials are too low. (c) There are not toxic compounds in this mixture. (d) The tests as performed cannot detect toxicity of these compounds.

Essentially no changes in the mixed function oxidase activity in the liver were induced by exposure to concentrated water. Interpretation of these negative results is difficult due to the complexity of the water mixture and the length of the experiment.

General observations in previous experiments with rats exposed to concentrated water indicated a resultant hyperactivity. Quantitative experiments done in the present study failed to reproduce this result in mice. This study should be considered a very preliminary behavioral assessment. There are more complex behavioral tests which have more relevance to human CNS toxicity.

Water concentrates were found to be mutagenic in previous studies using the dominant lethal mutation assay (5). In the current studies, the first experiment was positive the second was negative. The mean lethality of the control fetuses in the second experiment was double that in the first. Although the average fetal death in the experimental group was higher in the second trial than in the first, the difference was not significant. Wide experience has shown that the dominant lethal mutation test has several weaknesses. For example, studies performed on the same chemicals in different laboratories are not consistent. In addition, there is not always a dose-response relationship. Green and Springer mentioned that half of the 24 chemicals they tested failed to show a dose-response relationship (24). In an effort to resolve these conflicting results, mutagenicity studies with cells in culture were included in the current experimental program.

While short-term assays have been found useful for screening mutagenic and carcinogenic compounds, a parallel *in vitro* assay for general toxicity has not yet been established. Preliminary studies using human cells (WI38) were initiated in this project for the purpose for formulating a protocol for routine monitoring of water before dispatch. Water concentrates were found quite toxic to the cells, especially in the presence of a liver activation system. Surprisingly, the cells were more affected by the water at smaller doses than at higher doses in the presence of the liver microsomes. This effect could be the result of antagonistic effects on the cells of different compounds in the water. It is also possible that the microsomes are affected and indirectly affect the toxicity of the cells. The limited experimental data on this subject does not permit a conclusive explanation. Although several groups have tried to establish a biological monitoring system for water toxicity (25), there has not yet been a solution to this important problem.

Recently, several *in vitro* assays have been developed for the screening of possible carcinogens. Such tests quickly provide accurate assessment of the mutagenic and carcinogenic potential of the chemicals tested (26). Although there is no mechanistic evidence that mutagenesis is an essential part of carcinogenesis, a substantial amount of circumstantial evidence has been accumulated (13,27). Correlations were recently found between mutagenesis and transformation in the same tissue culture system (26,28). In the present study, no increase was noted in the number of revertant colonies above background in the bacterial mutagenicity test after exposure to water concentrate in the presence or absence of the activation system; however, a significant increase in the number of ouabain-resistant mutants was found.

The results presented in this study indicate that the concentrated recycled water caused increased rates of *in vitro* mutagenesis in mammalian cells, whereas no mutagenicity was indicated in the bacterial system. This apparent difference between bacterial and mammalian cells has been found before. It can be explained by differences in the genetic systems or other basic differences in cell structure and function. Recently, studies have been initiated by others to test the mutagenicity of organic fractions isolated from water (29) or purified compounds identified in water (30). Simmon, *et al.* (30) found that 34 percent of 71 compounds which were identified in water were shown to be mutagenic in the microbiological assays. However, since the selection of chemicals may have biased the results, they predicted that 10 percent of the chemicals in drinking water would be found to be mutagenic or carcinogenic.

Some preliminary work has been done in the past on the evaluation of water fractions for possible carcinogenic effects in animals (31-33). Contradictory results were reported. In this respect, it is important to mention the work done by Malaney, *et al.* (34) who showed that activated sludges failed to effect any significant removal of 27 proved (in animals) carcinogens (such as benzo(a)pyrene or propionolactone) by oxidation mechanisms within normal detection times.

Transformation of diploid cells *in vitro* is assumed to be a progressive process which may lead to the formation of malignant cells. One of the later events in this progression is the ability to grow on soft agar (35). While many rodent cell cultures have been found to exhibit *in vitro* transformation after exposure to chemicals (36), only limited success in transforming human diploid cells has been reported. The water concentrate in this study induced an increase in the number of WI38 colonies able to grow on soft agar, while potent carcinogens like benzo(a)pyrene and N-methyl-N-nitro-N-nitrosoguanidine failed to transform the cells. This may be explained by the presence of promoting agents in the water. Promoters have recently been shown to act under *in vitro* conditions (37,38). These results illustrate that the water, which contains low concentrations of certain carcinogens, may be more active when considered as a single sample due to synergistic effects of other compounds present in the total mixture.

An essential consideration is the relevance of such toxicological assays to the problem of human health. At present, there is not a good quantitative tool to relate experimental data to the human situation. It is important to note that TOC levels of the water in this study were only 100 times the concentrations present in many drinking water supplies. Such a difference between actual and experimental concentrations is considered minimal in toxicological assays. Barnes and Denz (39) calculated the minimum number of animals necessary to detect abnormal effects: To identify certain effects in 5 percent of the animals within 95 percent certainty, 58 animals must be studied; to identify effects in 1 percent of animals with the same degree of confidence, 295 animals are needed (close to the number used in this study). These pathological incidence rates, which represent the limit of sensitivity of the present toxicological tests, would, of course, be considered very high if found in the human population. It is generally accepted that these odds can be improved by increasing the exposure dose, as was done in this study, even though there is little data available on the relationship between dose and effect at critical doses.

In summary, while the water concentrate did not cause serious toxicological effects in many of the tests performed on the animals, mutagenicity and carcinogenicity properties of this water were demonstrated in tissue cultures. More mutagenic and carcinogenic in vitro tests should be performed on different water samples before these findings are extended to large scale, long-term animal bioassay programs.

REFERENCES

1. SCS Engineers, "Contaminants associated with direct and indirect reuse of municipal wastewater," EPA-600/1-78-019 report to the U.S. Environmental Protection Agency, Environmental Health Effects Research Series, Cincinnati, Ohio, 45268, March 1978, PB 280-482/AS.

2. Linstedt, K.D., E.R. Bennet, and J.N. English, "Research needs for the potable reuse of municipal wastewater," EPA-600-75-007, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268, December 1975.

3. World Health Organization, report of the International Working Meeting on Health Effects Relating to Direct and Indirect Reuse of Wastewater for Human Consumption, International Reference Center for Community Water Supply, Amsterdam, the Netherlands, Janurary 13-16, 1975.

4. van Rensburg, S.J., W.H.J. Hattingh, M.L. Siebert, and N.P.J. Kriek, "Biological testing of water reclaimed from purified sewage effluents," paper presented to the IAWPR International Conference on Advance Treatment and Reclamation of Wastewater, Johannesburg, South Africa, June 13-17, 1977, Prog. Water Tech., in press.

5. Gruener, N., "Long-term health effects of consuming renovated water," final report to the U.S. Binational Science Foundation, 1976.

6. Kopfler, F.C., W.E. Coleman, R.G. Melton, R.G. Tardiff, S.C. Lynch, and J.K. Smith, "Extraction and identification of organic micropollutants: reverse osmosis method," Annals of the New York Academy of Sciences 298 20 (1977).

7. Shuval, H.I., and N. Gruener, "Health considerations in renovating wastewater for domestic use," Environ. Sci. Techn. 7 600-604 (1973).

8. Wesson, L.G., "A modification of the Osborne-Mendel salt mixture containing only inorganic constituents," Science 75 339-340 (1932).

9. Nie, N.H., C.H. Hull, J.G. Jenkins, K. Steinbrenner, and D.H. Bent, Statistical Package for the Social Sciences, 1976, McGraw-Hill, New York.

10. Siegel, S., Nonparametric Statistics for the Behavioral Sciences, 1956, McGraw-Hill, New York, pp. 116-126.

References, continued

11. Ehrlich, K., E. Klein, and J.K. Smith, "Development of an on-line biological detector," final report to the U.S. Army Medical Research and Development Command, Contract No. DA-49-193-MD-1111, Washington, D.C. 20315.

12. Ames, B.N., J. McCann, and E. Yamasaki, "Methods for detecting carcinogens and mutagens with the salmonella/mammalian-microsome mutagenicity test," Mut. Res. 31 347-364 (1975).

13. Kuroki, T., C. Dravon, and R. Montesano, "Microsome-mediated mutagenesis in V79 Chinese hamster cells by various nitrosamines," Cancer Res. 37 1044-1050 (1977).

14. Warner, H.P., J.N. English, and I.J. Kugelman, "Wastewater treatment for reuse and its contributions to water supplies," EPA-600/2-78-027, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268, 1978, PB 280 145/AS.

15. Warner, H.P., J.N. English, and I.J. Kugelman, "The influence of municipal wastewater treatment on used water withdrawn for domestic supplies," presented to the annual convenstion of the American Water Works Association, Anaheim, California, May 1977.

16. National Interim Primary Drinking Water Regulation, EPA-570/9-76-003, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268, 1976.

17. Parodi, S., and G. Brambilla, "Relationships between mutation and transformation frequencies in mammalian cells treated in vitro with chemical carcinogens," Mut. Res. 47 53-74 (1977).

18. Clarke, G.D., M. Shearer, and P.J. Ryan, "Association of polyanion resistance with tumorigenicity and other properties in BHK/21 cells," Nature 252 501-503 (1974).

19. van Vuvren, L.J.R., M.R. Henzen, G.J. Stander, A.J. Clayton, "The full-scale reclamation of purified sewage effluent for the augmentation of the domestic supplies of the city of Windhoek," Adv. Water Poll. Res. 1, S.H. Jenkins, Ed., 1971, Pergamon, New York.

20. Ogilvie, J.L., "Wastewater reuse as a water resource - Denver experience," Proc. Workshop on Research Needs for Municipal Wastewater Reuse, U.S. Environmental Protection Agency.

21. Graesar, Henry J., "Water reuse: resource for the future," J. Amer. Water Works Ass. 66 575-578 (1974).

22. Tahal Water Planning for Israel Ltd. "Advanced treatment, groundwater recharge, and reuse of municipal wastewater," Dan Region Plant, Winter operation prepared for Mekorot Water Co. Ltd., Tel-Aviv, Israel, March 1976.

References, continued

23. Kritchevsky, D., "Modificiation of fiber of toxic dietary effects," Fed. Proc. 36:5 1692-1695 (1977).

24. Green, S., and J.A. Springer, "The dominant-lethal test: potential limitations and statistical considerations for safety evaluation," Environ. Health Perspec. No. 6, 37-46 (1973).

25. Morgan, W.S.G., and P.C. Kuhn, "A method to monitor the effects of toxicants upon breathing of largemouth bass," Water Res. 8:1 67-77 (1974).

26. Mishra, N.K., C.M. Wilson, K.J. Pant, and F.O. Thomas, "Simultaneous determination of cellular mutagenesis and transformation by chemical carcinogens in Fischer rat embryo cells," J. Toxicol. Environ. Health 4 79-92 (1978).

27. McCann, J., E. Choi, E. Yamaski, and B.N. Ames, "Detection of carcinogens as mutagents in the salmonella/microsome test: assay of 300 chemicals," Proc. Nat. Acad. Sci. USA 72:12 5135-5139 (December 1975).

28. Huberman, E., R. Mager, and L. Sachs, "Mutagenesis and transformation of normal cells by chemical carcinogens," Nature 264 360-361 (1976).

29. Loper, J.C., D.R. Lang, C.C. Smith, R.S. Schoney, F.C. Kopfler, and R.G. Tardiff, "In vitro mutagenesis and carcinogenesis testing of residual organics in drinking water," Aquatic Pollutants-Transformation and Biological Effects, Proceedings of the 2nd International Symposium, Amsterdam, September 1977, Perqammon Press Oxford, England, (in press).

30. Simmon, V.F., K. Kauhanen, and R.G. Tardiff, "Mutagenic activity of chemicals identified in drinking water," Second International Conference on Environmental Mutagens, Edinburgh, Scotland, July 11-15, 1977, pp. 249-258.

31. Hueper, W.C., and W.W. Payne, "Carcinogenic effects of adsorbates of raw and finished water supplies," Amer. J. Clin. Pathol. 39:5 475-481 (1963).

32. Ottoboni, A., and A.E. Greenberg, "Toxicological aspects of wastewater reclamation," J. Water Poll. Control Fed. 42:4 493-499 (1970).

33. Dunham, L.J., R.W. O'Gara, and F.B. Taylor, "Studies on pollutants from processed water: collection from three stations and biological testing for toxocity and carcinogenesis," Amer. J. Public Health 57:12 2178-2185 (1967).

34. Malaney, G.W., P.A. Lutin, J.J. Cibulka, and L.H. Hickerson, "Resistance of carcinogenic organic compounds to oxidation by activated sludge," J. Water Poll. Control Fed. 39:12 2020 (1967).

References, continued

35. Barrett, J.C., B.D. Crawford, and P.O.P. Tso, "The relationship between neoplastic transformation and somatic mutation," J. Cell. Biol. 70 233a (1976).

36. Freeman, A.E., H.J. Igel, and P.J. Price, "Carcinogenesis in vitro: 1. In vitro transformation of rat embryo cells - correlations with known tumorigenic activities of chemicals in rodents," In Vitro 11:2 107-116 (1975).

37. Mondale, S., D.W. Brankow, and C. Heidelberger, "Two-stage chemical oncogenesis in culture of C3H/10T1/2 cells," Cancer Res. 36 2254-2260 (1976).

38. Lankas, G.R. C.S. Baxter, and R.T. Christian, "Effect of alkane tumor-promoting agents on chemically induced mutagenesis in cultured V79 Chinese hamster cells," J. Toxicol. Environ. Health 4 37-42 (1978).

39. Barnes, J.M., and F.A. Denz, "Experimental methods used in determining chronic toxicity: a critical review," Pharmacol. Rev. 6 191-242 (1954).